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An Analysis of Toads of the *Bufo americanus* Group in a Contact Zone in Central Northern North America

Francis R. Cook

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AN ANALYSIS OF TOADS OF THE *BUFO AMERICANUS* GROUP IN A CONTACT ZONE IN CENTRAL NORTHERN NORTH AMERICA

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Abstract

Discriminant function analysis of 30 measured morphological characters was used to compare reference samples from Alberta to Prince Edward Island of 825 male *Bufo a. americanus* and 819 male *B. a. hemiophrys* and 59 female *B. a. americanus* and 118 female *B. a. hemiophrys*. Both analyses provided complete separation of the two taxa. When the resulting weights were applied to 2460 male and 225 female *Bufo* from southeastern Manitoba, an area excluded from reference samples, a relatively narrow zone of intermediate populations was defined. Separate analysis of three additional scored characters and of selected measured characters, alone or as ratios, followed the same pattern. Audiospectrograms of male breeding calls substantiated the presence of the intermediate populations and laboratory- and field-raised crosses between *B. a. americanus*, intermediate, and *B. a. hemiophrys* parents indicated some transformation success in all combinations.

Discriminant scores for *B. woodhousei fowleri* (19 males) and *B. w. woodhousei* (17 males) gave mean values different from the closest *B. a. americanus* or *B. a. hemiophrys* populations. Separate discriminant analysis comparing *B. a. hemiophrys* with *B. cognatus* (48 males) and *B. boreas* (60 males) showed complete separation of the taxa in areas of sympatry, and the morphological intermediacy of a single *B. a. hemiophrys* × *B. boreas* field-identified hybrid was confirmed.

The taxa *B. a. americanus* and *B. a. hemiophrys* are conspecific and lack apparent barriers to interbreeding with introgression indicated east and west of a narrow primary contact zone. Because of the narrowness of the primary zone of intermediate populations (about 16 km) and the distinctiveness of these taxa outside of this zone, they can be considered megasubspecies: *B. (americanus) americanus* and *B. (americanus) hemiophrys*. Two previously recognized subspecies, *B. a. copei* (northern Ontario and Quebec) and *B. h. baxteri* (Wyoming) are not here regarded as taxonomically distinct.

The intermediate zone between *B. a. americanus* and *B. a. hemiophrys* corresponds to the midpoint in transition between eastern and central herpetofaunas, elements of which are postulated to have been separated during a break in the transcontinental Boreal Forest during the Wisconsin, and perhaps earlier, glaciations.

Résumé

On a procédé à des analyses au moyen de fonctions discriminantes de 30 caractères morphologiques pour comparer des échantillons provenant de l'Alberta à l'île du Prince-Édouard et comprenant 825 *Bufo a. americanus* mâles, 819 *Bufo a. hemiophrys* mâles, 59 *B. a. americanus* femelles et 118 *B. a. hemiophrys* femelles. Les deux analyses ont révélé une séparation complète de ces deux taxons. L'application des résultats pondérés obtenus chez 2460 mâles et à 225 femelles *Bufo* du sud-est du Manitoba, une région non représentée dans les échantillons de référence, a permis de déterminer l'existence d'une zone relativement étroite où se trouve des populations intermédiaires. Une analyse séparée de trois autres caractères et d'une sélection de caractères mesurés isolément, ou sous forme de rapports, a été effectuée suivant le même schéma. Des audiospectrogrammes du chant nuptial d'un certain nombre de mâles ont confirmé la présence de populations intermédiaires, et l'élevage en laboratoire et dans la nature des produits du croisement de parents *B. a. americanus* intermédiaires et de *B. a. hemiophrys* a abouti à la métamorphose complète de spécimens issus de toutes les combinaisons.

Les résultats discriminants concernant *B. woodhousei fowleri* (19 mâles) et *B. w. woodhousei* (17 mâles) reflétaient des valeurs moyennes différentes de celles qu'a livrées l'analyse des populations les plus étroitement apparentées de *B. a. americanus* et de *B. a. hemiophrys*. Une analyse discriminante distincte visant à comparer des populations de *B. a. hemiophrys* à des populations de *B. cognatus* (48 mâles) et de *B. boreas* (60 mâles) a révélé une séparation complète des taxons dans les zones de sympatrie et a confirmé la position intermédiare d'un unique hybride de *B. a. hemiophrys* et de *B. boreas* qui avait été identifié sur le terrain.

Les taxons *B. a. americanus* et *B. a. hemiophrys* appartiennent à la même espèce et rien ne semble empêcher l'hybridation. Une introgression a été signalée à l'est et à l'ouest d'une étroite zone de contact primaire. Étant donné la faible étendue de l'habitat principal des populations intermé diaires (environ 16 km) et le caractère distinctif de ces taxons à l'extérieur de cette zone, ils peuvent être considérés comme des mega-sous-espèces: *B. (americanus) americanus* et *B. (americanus) hemiophrys*. Deux autres sous-espèces reconnues antérieurement, *B. a. copei* (nord de l'Ontario et du Québec) et *B. h. baxteri* (Wyoming) ne sont pas distinctes du point de vue taxinomique.

La zone intermédiaire entre les populations de *B. a. americanus* et de *B. a. hemiophrys* correspond au centre de la zone de transition entre les faunes herpétologiques de l'est et du centre, dont les éléments auraient été isolés par une interruption de la forêt boréale transcontinentale lors de la glaciation du Wisconsin, ou peut-être lors d'une glaciation antérieure.

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J.S. Bleakney was Curator of Herpetology at the National Museum during 1952–1958, and reference samples from this period as well as from Prince Edward Island in 1959 were made by him alone (1952–1953) or with my participation. Richard Otterdahl (1959), R.A. Henry (1960), M. Gordon Foster (1965), Stanley W. Gorham (1965), and C. Bruce Powell (1962, 1963, 1965) assisted my field programs which collected much of the remaining reference material. K.W. Stewart, Tom Vincent, Jim Johnston, Patrick Gregory, G. Viscotto, R. Merkel and J. Gilhen participated in portions of the southeastern Manitoba surveys. Andrew Kozlowski, F.W. Schueler, Wayne F. Weller, Ross MacCulloch, D.S. Redford and D. Summer made special efforts in obtaining additional reference collections used in this study. Kenneth R. Porter graciously made available his samples from the Laramie Valley.

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Frederick W. Schueler ran the major portion of the morphological data on the University of Toronto computer, contributed statistical insights and advice throughout, and searched out the geographic coordinates. Don E. McAllister, Curator of Ichthyology, National Museum of Natural Sciences, made the "ICHTHOS" computing facility available and also advised on statistical problems, as did Roger Green and Douglas Copeman.

Wendy Antoine, then of the Canadian Aquatic Identification Centre of the NMNS, converted a sea of crowded pencil notations into essentially flawless computer cards. D. Faber, L. Marhue and J. Fournier made this contribution possible.

Charlie Douglas, staff artist at the National Museum, painstakingly produced the views of *Bufo* included here.

Jim Johnston, as Curatorial Assistant in Herpetology from 1972, catalogued specimens, produced audiospectrograms from the original tapes, and recorded the sound variables used from them, ran deta on the "ICHTHOS" computer, drafted and photographed the graphs and maps included here, and aided in many other ways.

Marilyn Fisher typed difficult first drafts of the manuscript, and Jan McConnell and her staff at the National Museums' Word Processing Centre the several subsequent manuscripts, and Thérèse Lapierre made final corrections. Eleanor Fenton saw the manuscript through to publication, and Bonnie Livingstone provided her expertise on format. The Translation Bureau, Secretary of State, prepared the translation of the Abstract.

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corporated into the present revisions to its benefit but there remain areas of interpretation, particularly in taxonomy, where one or several of these reviewers would have strongly preferred a different conclusion. The retention here of my original conclusions implies no lack of awareness, respect or appreciation for their arguments.

The late E.B. Shelley Logier, formerly Curator of Herpetology of the Royal Ontario Museum, first stressed to me the lack of herpetological knowledge of central Canada and the need for intensive studies there nearly 40 years ago. This work is a direct result of those conversations.

Introduction

Five species of toads of the 187 (Gorham 1974) recognized in the nearly cosmopolitan genus *Bufo* (Amphibia: Anura: Bufonidae) have been recognized in northern North America (Conant 1975; Stebbins 1966). Three are broadly distributed: the American Toad, *Bufo americanus*, the Canadian Toad, *B. hemiophrys*, and the Northwestern Toad, *B. boreas*. These are largely allopatric and replace each other from east to west (Figure 1). North-south boundaries lie through eastern Manitoba and western Alberta. All are widespread in the boreal or montane forests which cover much of this region, but none invade tundra or true alpine conditions. They are abundant in the deciduous forests of the east *(B. americanus)*, the parkland and northern fringes of the grassland of the central portion *(B. hemiophrys)* and the arid interior valleys and coastal rainforest of the west *(B. boreas)*. Two additional species invade or approach the southern fringes of the region. In the east, Fowler's Toad, *B. woodhousei fowleri*, reaches its northern limit along the sandy north shore of Lake Erie in southern Ontario. Here and south to the Atlantic coastal plain it is geographically sympatric with, but largely ecologically separated from, *Bufo americanus*. In the central portion of the continent Woodhouse's Toad, *B. woodhousei woodhousei*, is allopatric to *Bufo hemiophrys* along a line from northern Montana to northern South Dakota (Figure 1). The Great Plains Toad, *B. cognatus*, which is widely sympatric with *B. woodhousei* over the interior of the continent south to northern Mexico, is narrowly sympatric with *B. hemiophrys* along the grassland southern fringe of its range in southeastern Alberta and southwestern Saskatchewan (Figure 1). A disjunct population of *B. hemiophrys* occurs in the Laramie Valley of Wyoming. In western Alberta and in British Columbia, the Yukon and coastal Alaska, *B. boreas* is the only toad, but to the south its range interdigitates with other species (e.g. *B. w. woodhousei* in Washington and further south).

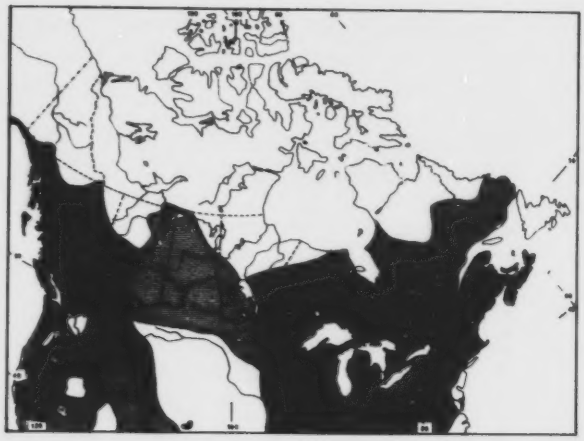
The literature on toads is voluminous (Blair 1972) and the evolutionary relationships, and many of the comparative aspects between species are extensively documented (osteology, chromosomes, electrophoretic patterns of proteins, venom constituents, and laboratory hybridization, among others). Blair (1972 and previous papers) has placed all the species considered here in his narrow-skulled (or thin-skulled) northern evolutionary line within the genus, and concluded that they represent three species-groups. *B. americanus, B. hemiophrys* and *B. woodhousei* are all included in the *americanus* group; *B. cognatus* and *B. boreas* each are placed in distinct groups. However, relatively little has been published on northern populations of these taxa, and their geographic variation and interactions within this region have been poorly documented or are unknown.

This study attempts to clarify the relationship and interaction between *B. americanus* and *B. hemiophrys*. It has been possible to examine a larger number of specimens than any previous authors had available from over the combined range of the two forms. This sample encompasses northern North America from Prince Edward Island to the eastern foothills of the Rocky Mountains in Alberta. The geographic variation of their populations over this vast area is compared to the variation in population samples obtained through transects of their contact zone in eastern Manitoba. Calls of individuals from intermediate populations are compared to those of both eastern *(B. americanus)* and western *(B. hemiophrys)* populations adjacent to the contact zone and the relationship of morphological variation and call variation is compared. Data on crossing success are less extensive and less satisfactory but it is the first attempt to field-rear tadpoles of crosses between these forms and compare their viability in different pond environments.

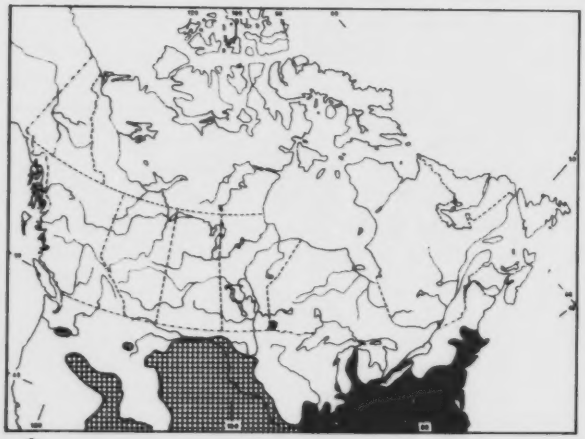
Also discussed are comparisons between the *B. americanus-hemiophrys* complex and samples of *B. w. woodhousei, B. w. fowleri, B. cognatus* and *B. boreas*.

Throughout the remainder of this paper I anticipate its conclusions and refer to the taxa previously called *Bufo americanus* and *Bufo hemiophrys* as *B. a. americanus* and *B. a. hemiophrys*.

Figure 1. The geographic distribution of toads in northern North America based on Conant (1975) and Stebbins (1966), with modifications and additions from National Museum of Natural Sciences (Herpetology Section) collections and Cook (1977).



Bufo a. americanus*, *B. a. hemiophrys and ***B. boreas*** replace each other across the continent from east to west, respectively. Note the narrow zone of sympatry between ***B. a. hemiophrys*** and ***B. boreas*** in central Alberta, and the disjunct population of ***B. hemiophrys*** in Wyoming (bottom of map, west of centre).



The ***Bufo woodhousei*** complex: ***B. w. woodhousei*** in the west and ***B. w. fowleri*** in the east. Compare with the top map for area of sympatry between ***B. w. fowleri*** and ***B. a. americanus*** in the east, for allopatry between ***B. w. woodhousei*** and ***B. a. hemiophrys*** in the central portion and sympatry between ***B. w. woodhousei*** and ***B. boreas*** in the west. Ecological separation occurs within the areas of geographic overlap.

Bufo cognatus. Compare with top and middle for areas of sympatry with ***B. a. hemiophrys*** and ***B. w. woodhousei***. There is some ecological separation between ***B. cognatus*** and the other species within their overlapping ranges.

Materials and Methods

Description of Areas Sampled

The primary vegetation zones of Canada have been discussed and mapped by Rowe (1959) and these are used on the base map showing position of reference samples of *Bufo a. americanus* and *B. a. hemiophrys* in eastern and central North America (Figure 2).

Forest region distributions depend on a variety of climatic, topographic, soil, and geologic variables and are, at least in part, a function of the combined effect of these on gross vegetation. Amphibian and reptile distributions are influenced by the same variables and often show some general agreement with vegetation zones (Savage 1960) though exact range limits for particular species vary as much as those of individual tree species. Forest regions are used here as a crude measure of climatic and other variables which affect toad distribution.

Southeastern Manitoba was most intensively sampled because it includes the contact zone between toad taxa that is central to this study. Figure 3 shows the collecting localities and vegetation types in this area. The southern tongue of the Manitoba lowlands section of Rowe (1959) is important in the following analysis. This is an area of change from predominantly coniferous trees of the Boreal and Great Lakes complex to aspen and grassland vegetation. At some localities (e.g. the Brokenhead River at Highway 1; Locality 35) the change is abrupt and can be readily appreciated in the field. However, various coniferous outliers exist to the west (e.g. at Birds Hill), and simple correlations of toad morphology and tree species cannot be readily made.

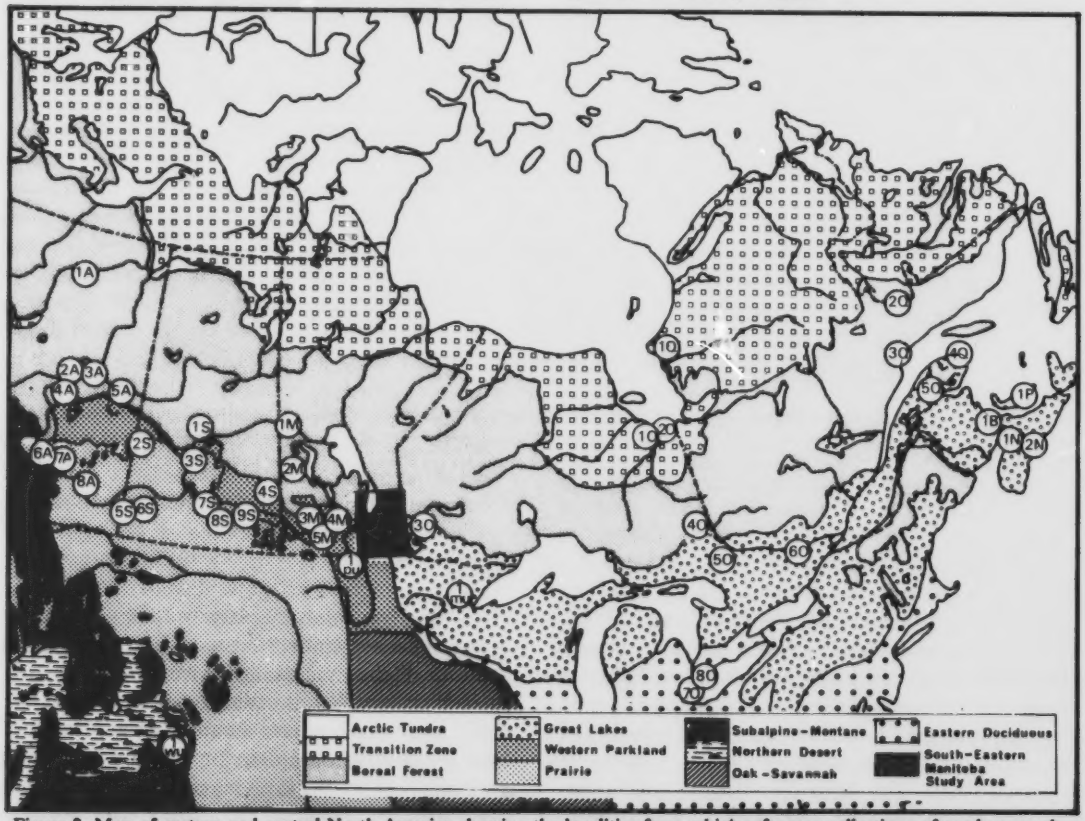


Figure 2. Map of eastern and central North America showing the localities from which reference collections of toads were obtained. These localities are numbered separately within each province or state and are listed in Appendix I with catalogue numbers for each collection examined. The forest regions shown are simplified from Rowe (1959) with southern extensions from Clarke (1973).

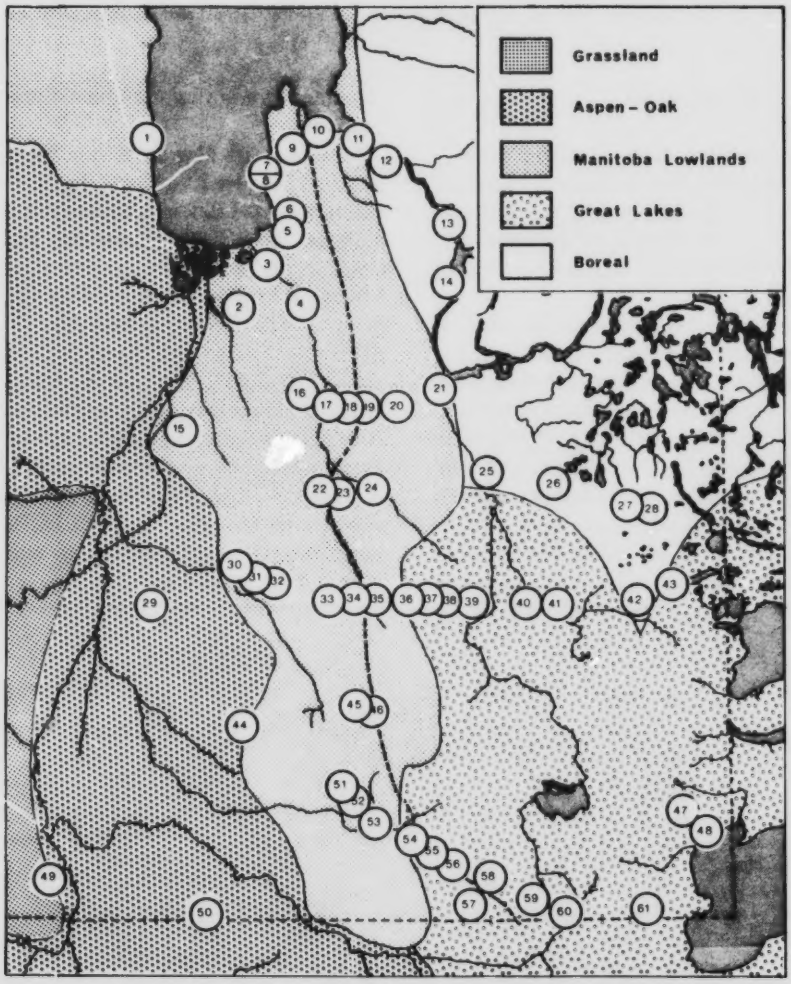


Figure 3. Map of southeastern Manitoba showing the localities from which collections of toads for analysis were obtained. Locality numbers are listed in Appendix I with catalogue numbers of specimens examined. The forest regions shown are simplified from Rowe (1959). The dashed line represents the mid-point of the contact zone between *Bufo a. hemiophrys* (west of line) and *B. a. americanus* (east of line) as discussed in the text.

Southeastern Manitoba has been modified by clearing for tillage and drainage for agriculture, primarily in its western portion (Warkentin 1967). The vegetational transition and area of contact between toad taxa features abundant beach ridges of glacial Lake Agassiz and is bordered on the east by peat and swamp and to the west by clay soils (Figure 4 in Elson 1967).

Löve (1959) and Shay (1967) have presented discussions of the post-glacial vegetation in this area, demonstrating the changes that have occurred over the past 12 000 or more years.

Field Surveys

Field work was conducted largely by motor vehicle along highways and rural roads. For this reason roadside ditches, ponds, lakes, and rivers were the main areas sampled. Breeding toads were of prime concern. These are here assumed to provide the best population samples for geographic comparisons because the individuals are known to be mature adults. However, additional samples not included here were also taken throughout the active season for amphibians and reptiles whenever

possible, in order to obtain tadpoles of various growth stages, metamorphosing individuals, and samples of juveniles and adults outside the reproductive season.

General survey collecting sites were chosen to provide a wide geographic sample. In Manitoba, transects were run across the southeastern corner of the province on the east-west network of public roads. Particular attention was given to intensive sampling where the putative zone of taxa contact seemed most obvious.

An eight-cell headlamp was used for illumination during after-dark collecting. At peak breeding, and usually at other times as well, this artificial light is apparently ignored by toads and they continue normal activity.

Attempts were made to obtain a minimum of 20 breeding males per locality, although this was not always possible. Much larger series (100 or more) were occasionally taken to provide a more robust sample for statistical analysis. Many Trans-Canada Highway localities were resampled to allow analysis for possible within- and between-year variation.

Generally, a large proportion of males utilizing a particular site for breeding could be expected to be present on a peak breeding night, but females tended to arrive over a more extended period though the breeding season, breed shortly after joining the chorus, and leave after depositing their eggs. This results in a few females relative to the numbers of males being available at any sampling period. In addition, females tend to come to the ponds in greatest numbers on a few peak nights of the breeding season, whereas males, though in reduced numbers compared to peak intensity, continue to call and be present over much of the breeding season. Because of their call, males are also more conspicuous. Juveniles were rarely present at the breeding site, but when present could be recognized as immature by size and lack of breeding condition.

Field notes on habitat, general topography and weather conditions, as well as relative density and behaviour of toads, were made for each locality at the end of each sampling period.

Specimens for preservation were usually processed within 12 hours of capture, but occasionally they were retained in dampened cloth bags under refrigeration for longer periods.

All were killed with ether, were injected with 10% formalin and individually tagged, then immersed in formalin of the same strength (Cook 1965a). Permanent museum catalogue numbers were assigned to each collection in the field, and individuals were given a subnumber within that catalogue number. For musuem storage, collections were transferred to 45% isopropyl alcohol.

Morphological Character Set

The following character set was devised primarily to compare *B. a. americanus* and *B. a. hemiophrys* on the basis of characters which appeared to differ between the two taxa or which showed variation of interest within each taxon. It was later applied to one sample of *B. boreas* and one of *B. cognatus* and to small samples of *B. w. woodhousei* and *B. w. fowleri* to compare their variation in these characters with *B. a. hemiophrys* and *B. a. americanus*. Additional characters could have been added which would have improved the separation in certain of these combinations.

Many of these characters, in the same or slightly different forms, have been used previously to differentiate *B. a. americanus* and *B. a. hemiophrys*. Blair (1957a), Wright and Wright (1949), Breckenridge (1944), Conant (1975) and others have presented comparative descriptions. Previous attempts at quantification have been made by Underhill (1961), to compare variation within populations of *B. hemiophrys* and between *B. hemiophrys* and *B. woodhousei* and *B. americanus*. Porter (1968) compared the Wyoming relict with samples from the continuous range of *B. hemiophrys*, and Henrich (1968) assessed populations from South Dakota which he showed to be intermediate between *B. hemiophrys* and *B. americanus*.

All measurements and coding states used in the present analysis were made after at least two years preservation. Every measurement was taken with the same model dial calipers to the nearest 0.1 mm by myself over a period of four years, and geographic sequence of measuring collections was random.

Lee (1982) has presented a statistical analysis showing significant variation in the measurements of *Bufo marinus* taken before preservation and after preservation periods of four and 14 months. Based on these results he questioned the validity of comparisons in morphometric studies that are based on preserved material. In my study the variation between collections is minimized by most collections having been processed similarly by J.S. Bleakney or myself (the most notable exception

is the RKP Wyoming collection which was stored in ethyl rather than isopropyl alcohol) though the relative periods in formalin or alcohol did vary by unrecorded amounts. The collections may have been in isopropyl alcohol long enough to have stabilized, and the general consistency of the results seems to indicate that preservation and measurement variations over time do not invalidate the analysis. Fresh measurements that I had taken for all specimens that I collected personally in the field were deliberately not used in the present study because of the obvious problem of shrinkage of individuals after preservation and the need to measure additional characters and to compare material for which fresh measurements by the same investigator were not available. Lee's (1982) point that preserved specimen measurements do not exactly represent the size or proportions of a living animal are valid and the caution inherent in his paper about overinterpretation of small differences has hopefully been intuitively avoided throughout the discussions presented here.

The following measurements were recorded for all specimens except for some *Bufo boreas* in which the cranial crests were not obvious, thus forcing the omission of all characters involving these structures.

Figure 4 depicts the measured characters and Figure 5 the ventral pattern variation. Figures 6 and 7 compare dorsal views of toads studied, and Figure 8 depicts ventral views of hind feet.

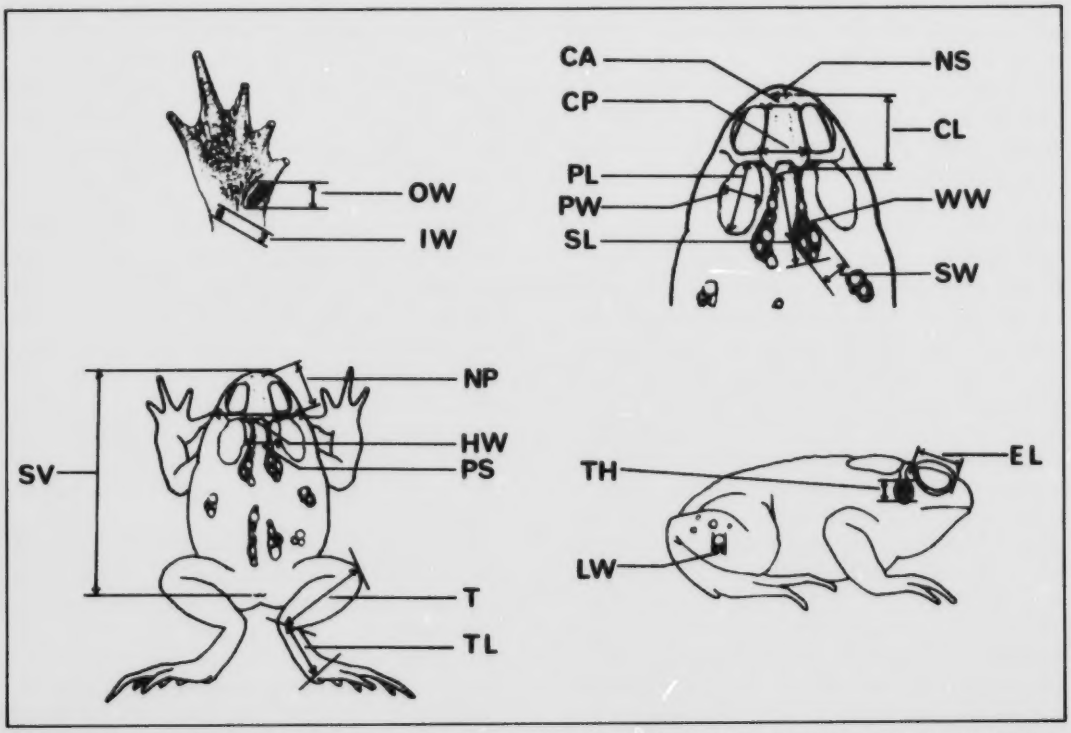


Figure 4. Diagrammatic views of a generalized toad showing underside of right hind foot, dorsal view of head and shoulders, and dorsal and lateral aspects of a whole animal.

CA: cranial crest: anterior width
CL: cranial crest length
CP: cranial crest: posterior width
EL: eyelid length
HW: head width
IW: tubercle width (inner metatarsal tubercle)
LW: diameter of largest wart on tibia
NP: nostril to parotoid
NS: nostril separation
OW: "spade" (outer metatarsal tubercle) width
PL: parotoid gland: length
PS: parotoid gland separation
PW: parotoid gland: width
T: tibia length
TH: tympanum height
TL: tarsus length
SL: spot length
SV: snout-vent length
SW: spot width
WW: wart width

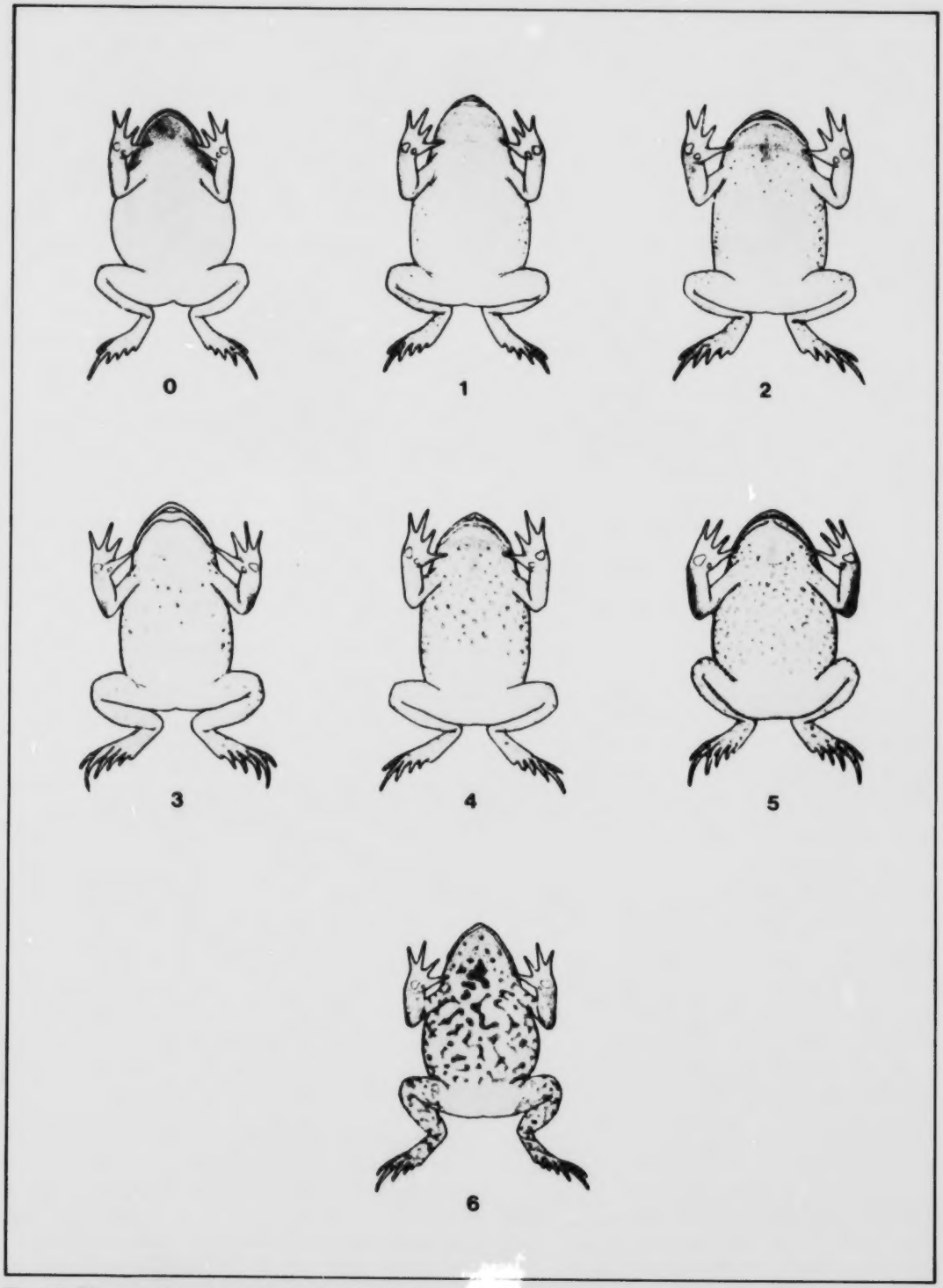


Figure 5. Diagrammatic venters of toads showing the range of ventral spotting and the scoring values (0–6) assigned (see text for definitions of scored conditions).

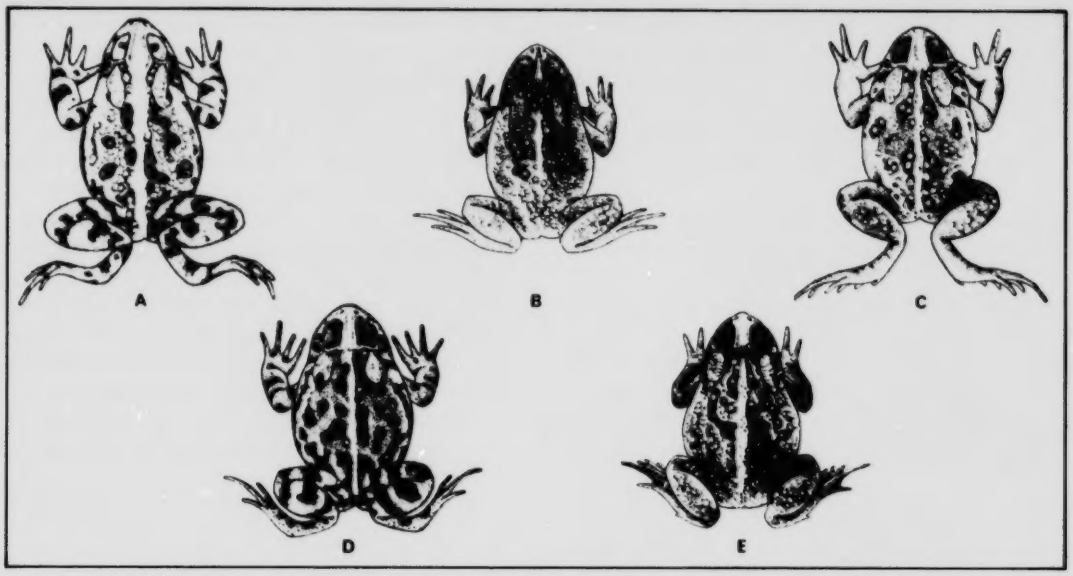


Figure 6. Dorsal views of selected male toads in the *Bufo a. americanus–B. a. hemiophrys* complex showing external structure and pattern.

A. *B. a. americanus*, NMC 14797-4, Whitetop Creek, Moosonee, Ontario
B. *B. a. americanus*, NMC 11795-3, 3 miles [4.8 km] west on Highway 1 of junction Highways 1 and 11, Manitoba
C. *B. a. americanus* x *hemiophrys*, NMC 8484-11, 17 miles [27.4 km] west on Highway 1 of junction Highways 1 and 11, Manitoba
D. *B. a. hemiophrys*, NMC 12217-1, 30 miles [48.3 km] west on Highway 1 of junction Highways 1 and 11, Manitoba
E. *B. a. hemiophrys*, NMC 8543-2, 10.5 miles [16.9 km] west and north on Highway 28 of St. Paul, Alberta

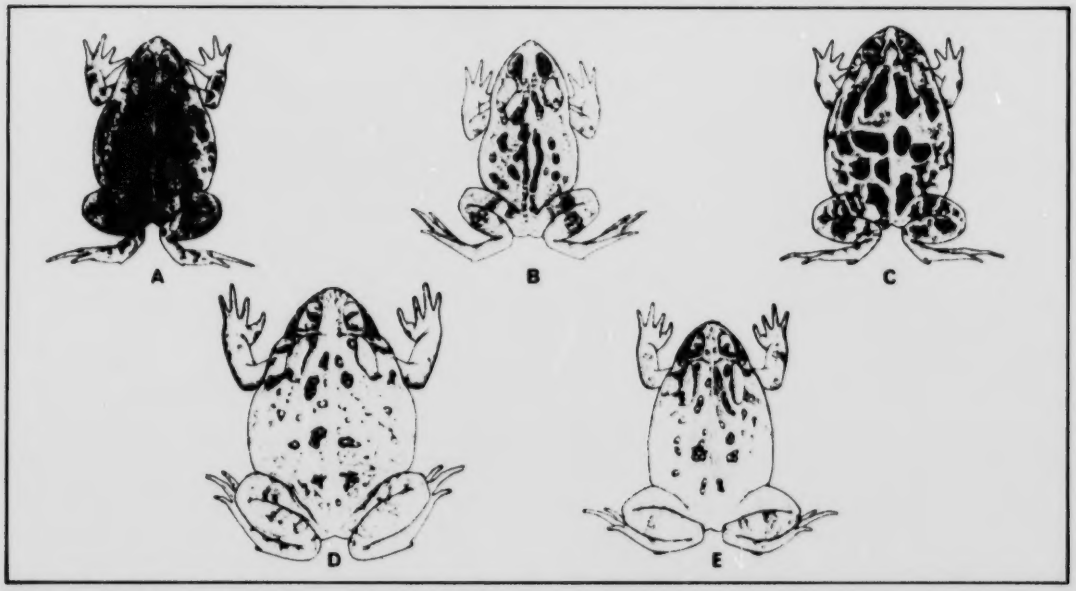


Figure 7. Dorsal views of other *Bufo* taxa and one hybrid.

A. *B. boreas*, NMC 17014, Mile 1.9 [Kilometre 3.1] on Kananaskis Lakes Road, Alberta
B. *B. boreas* x *B. a. hemiophrys*, NMC 8523, 2.4 miles [3.9 km] north on Highway 44 of Westlock, Alberta
C. *B. cognatus*, NMC 4168, 3.1 miles [5.0 km] east on Highway 1 of junction Highways 1 and 21, Saskatchewan
D. *B. w. woodhousei*, NMC 8591-6, 3.5 miles [5.6 km] SSE of Stockton, Rooks County, Kansas
E. *B. w. fowleri*, NMC 15972-10, Long Point, Norfolk County, Ontario

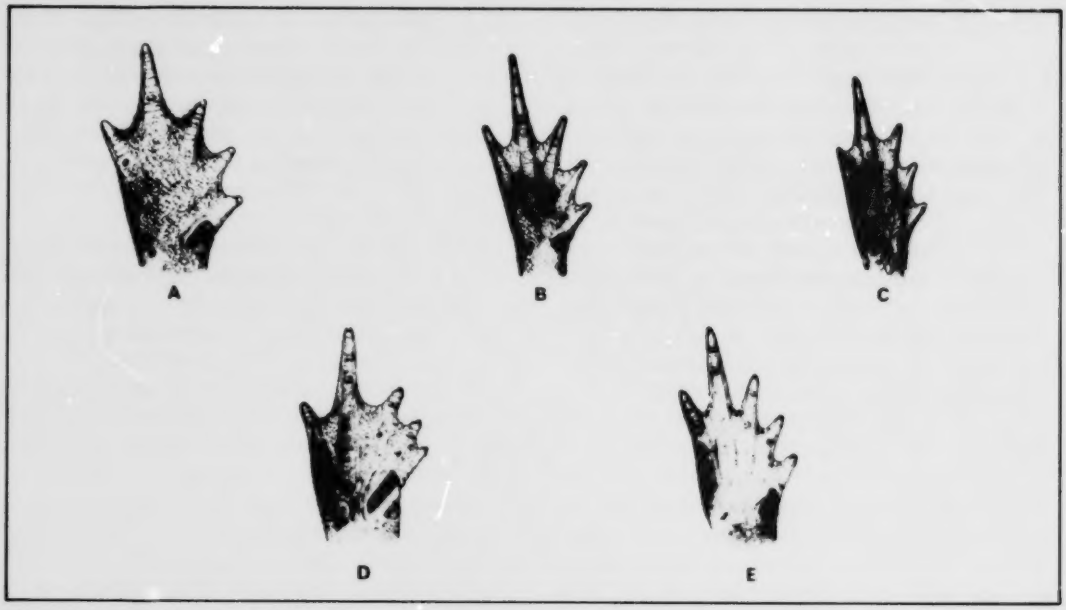


Figure 8. Ventral views of the right hind foot of selected toads.
A. *B. a. hemiophrys*, NMC 8521-35, 2.4 miles [3.9 km] north on Highway 44 of Westlock, Alberta.
B. *B. a. americanus*, NMC 7064-33, 10.5 miles [16.9 km] east on Highway 17 of North Bay, Ontario.
C. *B. boreas*, NMC 8530-33, 2 miles [3.2 km] west on Highway 2 of Athabaska, Alberta.
D. *B. cognatus*, NMC 7110-5, 8.3 miles [13.4 km] east on Highway 1 of Piapot (turnoff), Saskatchewan.
E. *B. w. fowleri*, NMC 15783-55, Long Point, Norfolk County, Ontario.

1. *Sex:* specimens were sexed by presence of nuptial pads and darkly pigmented vocal sac in males, both of which are absent in females. Since most collections analysed were from breeding populations this was generally adequate. Specimens less than the breeding size of the smallest mature male for that locality were designated as juveniles, and omitted from analyses.
2. *Snout-vent:* taken from the tip of the snout to the centre of vent, with the toad pressed as flat as conditions of preservation would allow.
3. *Nostril separation:* the distance separating the nostrils measured across the nose between their interior (medial) edges.
4, 5. *"Cranial crest" length, right and left sides:* measured from the nostril to the posterior edge of the crest. The anterior edge of the cranial crest merges with the bony structure of the nose so variously that the nostril was the best fixed point for anterior reference although this necessitates including more than the crest itself.
6. *Cranial crest type:* four arbitary categories were based on the difference between extreme *B. a. americanus* and *B. a. hemiophrys* and the intermediate condition.
   0) – a solid, filled-in boss caused by the cranial crests lacking a groove between them or having only a slight trace of a groove. The extreme *B. a. hemiophrys* condition.
   1) – crests parallel or divergent anteriorly with a bridge joining them across the back, and a distinct, but shallow, groove between them in the boss. Many *B. a. hemiophrys*.
   2) – crests variable, but always with either a trace of filling-in between them or a partial posterior bridge. Common in many individuals from hybrid populations.
   3) – crests distinct, no trace of filling-in between them and usually convergent anteriorly and strongly divergent posteriorly, but often hooked in slightly toward each other

at the extreme posterior. The typical *B. a. americanus* condition. Of the other toads analysed, *B. cognatus* and *B. woodhousei* were also scored in this category. The anterior convergence and posterior divergence is extreme in *B. cognatus*, and the crests usually less divergent in *B. woodhousei*. *B. boreas* has low indistinct crests if they are visible at all, and all crest characters were omitted for this species.

7, 8. *Nostril to parotoid, right and left sides:* the distance from the middle of the nostril to the anterior edge of the parotoid gland on both the right and left sides. A problem sometimes arose in defining the anterior edge of the gland, which may be somewhat indistinct especially in cases where it was pressed against the post-orbital crest or where a small wart was present between its anterior edge and the post-orbital crest.

9. *Cranial crest, anterior width:* cranial crests are raised bony structures that lie between the eyelids on the top of the head. They are more or less complete from the anterior edge of the eyelids to at least their posterior edges (and often beyond, especially in *B. a. americanus*). In *B. a. americanus* they tend to slightly converge anteriorly and flare widely posteriorly; in *B. a. hemiophrys* they may be more-or-less parallel (sometimes slightly bulging or contracting in the middle) but generally diverge anteriorly and slightly or strongly converge posteriorly. The anterior width of the crests was measured across their exterior (lateral) edges at approximately the level of the anterior edge of the eyelids. Some difficulties in precision are inherent, as the crests, particularly in *B. a. hemiophrys*, merge with the bony crest of the nose, and the latter begins to spread just anterior to the reference point used. In *B. a. americanus* the nose crest is less prominent and the anterior portion of the crest is more distinct. Despite the potential for inconsistencies in measurement, the crest differences are sufficient to make this measurement one of the most apparent distinctions between taxa.

10. *Cranial crest, posterior width:* taken across the posterior lateral cranial crest edges, just anterior to their junction with the post-orbital crest or (in many *B. a. hemiophrys)* just anterior to the post-orbital "wing", a short right-angled spur more-or-less in the plane of the post-orbital crest.

In *B. a. hemiophrys* the crests often do not extend past the post-orbitals and therefore a more posterior measurement, which would have accentuated the crest divergence in *B. a. americanus,* was not possible if consistency in measuring the two taxa was to be maintained.

11. *Post-orbital crest:* three arbitrary categories were based on the relative prominence of the bony crest that extends behind the eyelids, from the lateral edges of the cranial crests, at or near their posterior ends, to the tympanum.

0) – post-orbital crest absent or nearly so (not including in this evaluation the short spur on the lateral edge of the cranial crest present in some *B. a. hemiophrys*).

1) – an intermediate condition between 0 and 2, where the crests are slightly evident, either low and relatively indistinct throughout or else broken on one or both sides.

2) – post-orbital crests elevated and generally distinct.

12. *Eyelid length:* the anteroposterior length of the protruding pad of skin above the eye.

13. *Head width:* taken across the posterior portion of the head of the level at the tympani and between their upper edges. There are bony ridges along the upper edges giving a relatively solid structure to measure against.

14. *Tympanum diameter:* the greatest diameter of the eardrum, usually taken on a vertical plane.

15–18. *Parotoid gland, right and left sides:* the parotoid gland lies roughly over the shoulder of the toad and is generally an elongate, somewhat bean-shaped or oval gland. Generally, the margins are distinct in *B. a. americanus* but in *B. a. hemiophrys* they may be indistinct, especially at the lateral and posterior edges. Adjoin-

ing warts sometimes merge with the borders of the parotoid gland, particularly posteriorly, and make definition of gland boundaries difficult. The criterion applied for wart inclusion or exclusion in measurement of the parotoid was to omit them whenever possible, that is, whenever they had any appearance of being a distinct wart, whether or not they did actually touch the parotoid gland. Often the left and right parotoids were unequal in size. Separate measurements were taken for left and right sides.

15, 16. *Length:* the longest axis of this gland.

17, 18. *Width:* taken at right angles to length, at the widest point.

19. *Parotoid separation:* taken between the inside edges of the two parotoid glands at the point where the left and right glands approach nearest to each other.

20, 21. *Spot length, right and left sides:* a measure of the size of markings on an individual toad and the various relations of this to wart size and number. The spot that is usually present on the back at the level of the posterior edges of the parotoid glands and adjacent to the midline on each side was chosen. In rare cases where this was absent the nearest spot to its position was used. There is considerable individual variation in spot size and shape in both species and in intermediates. The spot is most frequently round or oval but irregular shapes also occur and there is a variable degree of fusion of adjacent spots. Any complete separation, no matter how narrow, was used to define the boundary of this from adjacent spots, but generally constrictions of varying degrees were ignored. In some cases, the spot would be quite regular but in others it would extend far beyond the usual position. Occasionally a spot was continuous far posteriorly on the back and sometimes anteriorly to the post-orbitals. Only rarely did spots cross the midline. Spots were usually aligned anteroposteriorly, parallel to the midline. In all cases this measurement was taken as the longest axis regardless of spot orientation.

22, 23. *Spot width: right and left sides:* the widest width, that is, the widest portion of the shorter of the two axes, subject to similar variation to that described in the above section.

23. *Tibia:* the length of the bone formed by the fusion of the right tibia and fibula in toads, measured from knee to heel with the leg flexed, as it is in most preserved specimens. The measurement taken will be slightly larger on preserved material than on the cleaned bone itself because of inclusion of adherent skin. Minor additional error may be introduced by difficulties in completely flexing the leg in preserved material and inadvertently including some of the adjoining articulation of other leg bones. With care this can be largely avoided. The right tibia was measured in all but a few specimens in which it was obviously deformed, broken, or missing. In such cases the left was used instead.

24, 25. *Wart width, right and left sides:* the width, or largest diameter, of the largest wart in the above spot. Generally warts are more or less circular, but in the rare instances of elongate warts the width was taken across the wart in the same axis as the width of the spot.

26, 27. *Number of warts per spot, right and left sides:* the number of warts present in each spot defined above. Difficulties due to apparent merging or fusing of adjoining warts were resolved by counting as distinct warts each one defined by a separate peak or each portion separated by a distinct crease. Lack of complete separation of warts is most common in *B. a. americanus*.

29, 30. *Diameter of the largest wart of the tibia, right and left tibia:* the longest dimension of the largest wart on the upper surface of the tibia. If two obvious peaks were present the wart was regarded as fused from two warts and the measurement taken across the wart so as to measure only the larger one. However, elongated warts which had no obvious peaks were considered one wart and the measurement taken on the long axis.

31. *Tarsus:* measured from a point on an imaginary line across the posterior (proximal) edges of the metatarsal tubercles (see below) to the "heel"—the junction of the tarsus and tibia. There is a certain

amount of measurement variation introduced because of difficulties in flexing the tibia clear of the end of the tarsus in some preserved specimens.

32. *Spade width:* the projection used for digging, largest of the two metatarsal tubercles on the outer edge of the tarsus, measured across its base from the inner (medial) corner to the outer (lateral) edge of the peak. This measurement is an attempt to express the effective digging edge width. It may not, as taken, express the full width of spade but is chosen because of the clarity of its reference points.

33. *Tubercle width:* the smaller, medial, metatarsal tubercle on the tarsus measured across its base.

34. *Venter:* a scoring system based on Blair (1943) for the relative amount of the undersurface covered by dark markings (see Figure 5).
    0) - no markings on the ventral surface (exclusive of the throat)
    1) - spot in the pectoral region only
    2) - spots covering the chest region only
    3) - spotting over the anterior 1/3 of the venter
    4) - spotting over anterior 2/3 of venter
    5) - spotting over the entire venter
    6) - reticulated pattern over the venter
    This system, unfortunately, excludes much additional variation in ventral markings. The spotting varies in intensity from dark to faint. However, because the variation due to fading in preservative could not be distinguished from natural variation no attempt was made to quantify intensity for geographic analysis. There are also differences in the size of the irregular ventral spots and in their relative density on the undersurface that were also ignored in this analysis.

Some additional characters, found useful by previous authors in comparing variation within or between these taxa, such as coloration (Gaige 1932; Ashton, Guttman and Buckley 1973; Conant 1975, to differentiate *B. a. copei*), width of mid-dorsal stripe, height of boss, ulna length (Porter 1968, to differentiate *B. h. baxteri*), parotoid distinctness (Breckenridge 1944; Henrich 1968), appearance of warts, snout length, head length, pattern of dorsal blotches (Henrich 1968), snout length and inter-orbital distance (Underhill 1961) were not included in the present analysis because of difficulties in objectively measuring or evaluating them, or because the variation they describe is accounted for by a different approach here. Of the omissions, coloration is the most serious because obvious differences are apparent between the two taxa. However, intermediates show such a complete gradation that an attempt at objective categories was abandoned. The character is useless with preserved material because of fading.

Call Analysis

During the 1969 and 1970 field seasons, tape recordings of breeding calls of individual male toads were made on a Nagra III tape recorder. A hand-held omni-directional microphone (Electro-Voice model 635) proved adequate because in virtually all cases toads could be approached and the microphone held within a few centimetres of the calling individual, effectively screening out adjacent members of the chorus. Occasionally adjacent toads were transferred to another portion of the breeding pond when they were too close to the individual being recorded.

An effort was made to obtain at least four complete, clear calls from each individual recorded. Most individuals were recorded at the ponds along the Trans-Canada Highway transect. In 1969 efforts were concentrated along the latter transect from about 3 miles [4.8 km] west to about 31 miles [49.9 km] west of the junction of Highway 1 and Highway 11 along Highway 1. Only the single transect was monitored for intermediate calls because of the time required to obtain individual calls. The assumption was made that it was potentially more informative to have many individuals along one complete transect than a few from each of several transects.

In 1970 call sampling was augmented on this transect and localities west in the range of *B. a. hemiophrys* to The Pas, Manitoba, and east in the range of *B. a. americanus* to Oxdrift, Ontario, were also sampled.

The body temperature of the recorded toad, as well as adjacent water and air temperatures, was taken immediately after recording was completed. Initially, body temperatures were taken by inserting the probe into the mouth and into the vent but it soon became apparent that the difference between temperatures taken by the two methods was essentially negligible and the first method was

abandoned. Temperatures were taken with a Yellow Springs single channel tele-thermometer (0°–50°C) equipped with a thermistor probe of small flexible vinyl (YSI Probe 402). The latter was inserted through the vent into the body and the temperature read as insertion was completed. Every effort was made to insert and read the temperature as soon as the animal was captured in order to minimize the effect of struggling while being held (and thus transferring human body heat). In general this proved practicable.

Individual toads were tagged (by a numbered tag tied to the right hind leg) after recording and the tag number read with temperature, date and locality directly on to the taped call for that individual. The number read was subsequently used for the sub-number in the series in which that toad was preserved, thus providing a means of identifying the individual preserved toad with its call and allowing comparisons between morphological parameters and call parameters.

Tapes were analysed in the laboratory on a Sona-Graph 6061A 85-8000 cps spectrum analyser manufactured by Kay Electronics, Pine Brook, New Jersey. The Sona-Graph produces audiospectrograms of a selected segment of a call. The segment used in the present analysis was chosen from the approximate mid-point of one uninterrupted call for each individual, after test samples of several calls each from selected individuals indicated no difference between calls for the variables considered. Zweifel (1968:271) also noted, in a study of *B. a. americanus* and *B. w. fowleri*, the same lack of difference between calls given by the same individual. The narrow band (45 cps filter) gave sharp enough resolution of pulse rate for most *B. a. americanus* and for *B. a. hemiophrys* at low temperatures, but wide band displays were necessary to distinguish pulses in *B. a. hemiophrys* recorded at warm temperatures. The number of pulses in a one second interval were counted directly from the audiospectrogram produced. The mid-point of the dominant frequency band was measured by means of a section to the nearest 100 cps. This is produced by the Sona-Graph as a graphic representation of the relative amount of energy found at different frequency levels at a selected point in a recording. These sections were generally taken about halfway through a call.

All calls recorded by a given individual were timed by a stopwatch and the mean duration of the call over the sample determined.

Computer Analysis of Data

All measurements were transferred to computer punch-cards, with two cards required for the data from each specimen. Analysis was run on the IBM system 370 at the University of Toronto Computing Centre.

The discriminant analysis program for two groups used for comparison of *B. a. americanus* and *B. a. hemiophrys* was programmed at the University of Toronto by D.M. Power, November 1967, revised December 1970 and December 1972. The procedure for this program had been taken from Anderson (1966). Discriminant function analysis requires the use of two groups chosen *a priori*. This was met by excluding all samples from southeastern Manitoba, well to either side of the area of peak interaction noted in field sampling. The reference samples were all collections east and west of this area, chosen therefore on geographical origin without regard to morphology. The weights produced by the analysis were then used to produce scores for the unknowns from the excluded area. Scored characters were omitted from the discriminant analysis and males and females were run separately on the remaining 30 characters (all representing measured values except for two counts—the number of warts per spot on the left and right sides). Because the computer would accept only a maximum of 500 specimens in each reference sample with this many characters used, and over 800 were available in each of the reference samples (819 *B. a. hemiophrys* and 825 *B. a. americanus*) for males, the program was set up to run every other specimen in one run for each taxon and then re-run a second time with the alternate specimens. Each run compared approximately one-half the sample of one taxa with one-half the sample of the other. Group I included 412 *B. a. americanus* and 409 *B. a. hemiophrys*, Group II consisted of 413 *B. a. americanus* and 410 *B. a. hemiophrys*. The two sets of discriminant weights were then averaged and the resultant weight for each character was applied to the individual scores for all *B. a. americanus* and *B. a. hemiophrys* in the reference samples and to 2460 male *Bufo* from southeastern Manitoba. Means, variances, standard deviations, and standard errors were calculated for each collection. In addition, histograms of the individual scores for each collection were generated.

The same program was run for females. The smaller female samples (59 *B. a. americanus* and

118 *B. a. hemiophrys*) were included on one computer run and the resulting weights applied to 225 females from southeastern Manitoba.

A sample of immature individuals proved too small to yield significant results. Although the taxa were distinctive when compared, the number of characters exceeded the number of specimens in one reference sample.

Programs for discriminant analysis comparisons of *B. a. hemiophrys* vs. *B. boreas* were also run in the University of Toronto program. Additional discriminant analyses involving morphological comparisons of *B. a. hemiophrys* vs. *B. cognatus* and the call comparison of *B. a. americanus* vs. *B. a. hemiophrys* were run on the National Museum of Natural Sciences, Ichthyology Section, Hewlett-Packard 9830 mini-computer "ICHTHOS" using Program 29: Hotellings T^2 and discriminant function for two groups from Davis (1971: 284–291) as programmed by D. E. McAllister.

Analysis of call variables and a discriminant function involving these were also computed on "ICHTHOS" (McAllister, Murphy and Morrison 1978) as well as the correlation of the discriminant function thus obtained for call and the one previously generated on morphological characters for *B. a. americanus, B. a. hemiophrys*, and intermediate populations.

Hybridization Experiments

During 1969 calling males and gravid females were collected for mating experiments from choruses along the Trans-Canada Highway transect through the interbreeding zone. The localities used were:

(1) 31–29 miles [49.9–46.7 km] W. on Highway 1 or junction of Highways 1 and 11: *B. a. hemiophrys*
(2) 17 miles [27.4 km] W. on Highway 1 of junction of Highways 1 and 11: intermediate population.
(3) 3 miles [4.8 km] W. on Highway 1 of junction of Highways 1 and 11: *B. a. americanus*

A total of nine crosses with three replicates of each (27 sets) were attempted.

female		male
americanus	×	*americanus*
americanus	×	*intermediate*
americanus	×	*hemiophrys*
intermediate	×	*intermediate*
intermediate	×	*americanus*
intermediate	×	*hemiophrys*
hemiophrys	×	*hemiophrys*
hemiophrys	×	*intermediate*
hemiophrys	×	*americanus*

Males and females were collected the same night from all three localities and paired in selected combinations in four- or 10-litre jars in pond water from the locality where the female had been collected. Mating and egg laying usually took place overnight but occasionally was delayed until the next day. If it did not occur within 48 hours the cross was repeated with another pair.

Two hundred eggs were counted out from the batch laid by each female of a successful cross, and divided into four lots of 50 eggs each. One set of 50 eggs from each cross was raised in the laboratory, one of each other lot was placed in a container in each of the three sites from which the adults had been collected. Laboratory crosses were raised in dechlorinated water in shallow plastic pans, field crosses were initially placed in circular petri dishes, each with a piece of their lid removed and replaced by plastic screening to permit water circulation. After hatching, the tadpoles were released into open plastic rearing cages made from plastic screening with a framework of plastic rods. Each unit was approximately 120 × 120 × 30 cm and each included four 30 × 30 × 30 cm compartments screened on the sides and bottom but open at the top. They were placed in the water at the edge of the ponds and submerged to approximately half their height. Receding pond levels due to evaporation during the period from hatching to transformation of the tadpoles and level rises after occasional heavy rains necessitated moving the units to compensate for these fluctuations in water levels. Initially, the eggs were checked at daily intervals, but later in tadpole development containers were checked at two-day intervals, until the last tadpole had metamorphosed. Tadpoles were fed Tetramin, a commercial "fish food" preparation that does not unduly contaminate water. The water in laboratory pans was changed whenever it appeared fouled but this was not necessary in field cages. Dead eggs and tadpoles were removed and noted at each inspection. Part way through the experimental period a disaster occurred at the "*americanus*" site when all rearing cages were removed from the water and placed neatly at the roadside by person or persons unknown. The majority of tadpoles then desiccated due to exposure to air before the next regular inspection, thus terminating these crosses.

Results

1. Field Surveys

Field work in eastern Manitoba in the breeding seasons of 1968, 1969, and 1970 revealed no strong differences in breeding times, either in the initiation, peaks, or termination of calling between *B. a. americanus*, *B. a. hemiophrys* or intermediate populations. *B. a. hemiophrys* localities tended to be in more open sites, and the air and water temperatures dropped there more rapidly on cool evenings, resulting in *B. a. hemiophrys* choruses tapering off or stopping earlier than *B. a. americanus* choruses.

The general pattern evident in the 1968–70 field seasons in eastern Manitoba was an initial emergence from hibernation and arrival of an occasional individual at the breeding site as early as 19 April but as late as early May. The earliest calling was 24 April in 1969, with the earliest amplexed pairs and eggs 6 May the same year. The first good choruses were heard 13 May 1968, 14 May 1969, and 17 May 1970, indicating that an early or late spring does not alter the time of the initial peak of breeding by more than a few days. It was common in all years to find good choruses on warm nights from mid-May through to early June and for calling to gradually taper off with a few persistent individuals calling as late as the first week in July. A few small choruses were found in middle and late June in 1968 but the bulk of the series from breeding ponds were taken earlier (see list of specimens examined in Appendix I for dates of strong choruses indicated by size of collections). A similar pattern emerges when the collection dates of breeding *B. a. hemiophrys* from throughout its range are examined. One large collection taken later in the year (60 specimens from the Spruce Woods Forest Reserve 20–21 July 1960) is a post-breeding sample from toads along a sandy road. All large breeding samples are from the latter half of May or early June.

It is notable that there is a much greater variation in the dates for *B. a. americanus* breeding series, especially the series (NMC 6916) obtained adjacent to Rondeau Park in southern Ontario on 17 April 1963. In the milder climate of southwestern Ontario toads apparently may reach full chorus as much as a month earlier than is possible in eastern Manitoba or the Prairie Provinces in general. In eastern Manitoba, there was no consistent pattern during this study of either *B. a. americanus* or *B. a. hemiophrys* beginning calling appreciably before the other, or continuing later. The latest chorus sampled was of *B. a. hemiophrys*-like individuals (Grand Beach Provincial Park) 21 June 1968 but there were *B. a. americanus* choruses at N.W. Angle Forest Reserve a few days earlier, 16–17 June 1968. In both cases the proximity of the breeding localities to large lakes may have retarded the start of the breeding and prolonged it in comparison to other localities.

Bufo started to metamorphose in early July in eastern Manitoba. The earliest noted was 2 July 1969. This coincided with the cessation of calling from the last few persistent males and represented roughly a month and a half for development after the initial peak breeding. The latest tadpoles were noted 5 August 1968, but most had transformed by the last week in July, representing a two–three week span of peak transformation that roughly corresponded with the span of most active breeding.

Breeding ponds for both *B. a. americanus* and *B. a. hemiophrys* in eastern Manitoba and elsewhere are variable. Artificial dugout ponds, roadside ditches, and shallow quiet shores of rivers and lakes are common breeding sites. A fairly wide area of shallow water seems to be preferred. The breeding sites for *B. a. hemiophrys*, being mainly in aspen parkland and prairie, are in more open surroundings due to the general habitat, and no conclusions can be drawn on any difference in site preference. In general, the same types of breeding sites seem to be chosen by the two taxa.

In 1970 a simple mark-recapture program was carried out at the intermediate (contact zone) locality 17 miles [27.4 km] west of the junction of Highways 1 and 11. Fifty-one male toads were marked and released between 17 May and 11 June. An additional 23 males were collected 18 May from this locality and preserved. Forty-nine recaptures of 28 individual toads were made. A total of 16 females was also taken during the same collecting period. Some males remain at the chorus through the peak breeding period although unmarked individuals may be arriving throughout. Four individuals marked and released 18 May were

recaptured on each of 22, 23, and 24 May and three others were captured on two of those nights. There was no evidence of an uneven distribution of morphological types between early and later samples. A simple population estimate gives figures of 42, 47 and 64 male toads for 23 May, 24 May, and 5 June, respectively.

2. Analysis of Morphological Variation for Reference Samples

a. Discriminant Analysis of Males

The two discriminations run on alternate specimens from the combined reference sample gave the weights or character coefficients shown in Table 1. A mean discriminant weight for each character was produced by averaging them (Table 1) and these mean weights used to compute discriminant scores for each individual.

The size of the weights reflect in part the relative importance of the characters considered in separating the taxa.

The relative effectiveness of the weights is dependent on the number of units an individual character varies over, and the separation between the taxa within this character. A method of expressing the general distance between taxa is the "Mahalanobis generalized distance" computed by finding the separation between the mean discriminant scores of the taxa. The contribution of each character weight to this value can be obtained by multiplying the weight by the difference between

Table 1. Discriminant weights given each character in two analyses (Groups I and II) of male reference samples of *Bufo a. americanus* and *B. a. hemiophrys*, and the mean weight of the two groups used in computing discriminant scores for individual males, and the weight contribution to the Mahalanobis generalized distance between the combined groups.

	Weight Group I	Weight Group II	Mean Weight	Mean Contribution
Snout-vent length	+0.16522861	+0.18881679	+0.177023	+0.9242126
Nostril separation	−8.1142540	−8.586230	−8.350242	+2.5555703
Cranial crest length (right)	+0.76029348	+3.2909012	+2.025597	+3.5536756
Cranial crest length (left)	+3.6722383	+0.50351000	+2.087874	+3.6579865
Nostril to parotoid (right)	+0.41164047	−0.78529143	−0.186825	−0.2177316
Nostril to parotoid (left)	−0.59715325	+1.0789080	+0.838030	+0.9472763
Cranial crest width (anterior)	−4.7215214	−4.5984650	−4.659993	+0.2671204
Cranial crest width (posterior)	+8.3745794	+8.6095295	+8.492055	+21.7769295
Eyelid length	−3.5560398	−3.2611437	−3.408592	−1.4677809
Head width	−2.0142698	−1.8766050	−1.945437	−1.3364795
Tympanum diameter	−0.65865707	−0.21684265	−0.437750	−0.3360502
Parotoid length (right)	+0.073451102	+0.31809098	+0.195771	+0.2395683
Parotoid length (left)	−0.48348296	−0.37021834	−0.426351	−0.4372118
Parotoid width (right)	+1.0284834	+0.13527966	+0.581882	+0.0483071
Parotoid width (left)	−0.89462119	+0.41438520	−0.240118	−0.0093115
Parotoid separation	−3.0141783	−2.6087561	−2.811467	+4.1812830
Spot length (right)	−0.034990408	+0.036637843	−0.000824	−0.0008016
Spot length (left)	+0.055400848	+0.025961559	+0.040681	−1.0455871
Spot width (right)	−0.37663031	−0.18416786	−0.280399	+0.0474776
Spot width (left)	−0.76379973	−0.31844270	−0.541121	+0.0950692
Wart width (right)	+3.37714357	+1.0056877	+2.388562	+1.8619682
Wart width (left)	+3.5572758	+1.7886143	+2.672945	+2.1319337
No. warts per spot (right)	−0.090008795	−0.10848355	−0.099246	+0.1581416
No. warts per spot (left)	−0.098180711	−0.18084419	−0.139512	+0.2189280
Tibia length	−1.1040401	−0.79755020	−0.950795	−2.1168822
Diameter of largest wart on tibia (right)	+0.29262280	−0.93891734	+0.615770	+0.5780064
Diameter of largest wart on tibia (left)	+1.0001144	+1.13967094	+1.198411	+1.1220402
Tarsus length	+4.0203476	+4.3927183	+4.206533	+10.3077514
"Spade" width	−6.1017218	−6.1168079	−6.109264	+1.9970081
Inner metatarsal tubercle width	+3.0812159	+0.45624775	+1.768732	+0.6493347

Mahalonobis generalized distance (mean): 51.3517513

the means of each taxa for that character. This gives the *weight contribution* (to the Mahalanobis generalized distance) and the sum of these contributions is equal to the Mahalanobis generalized distance (D.E. McAllister, personal communication). This weight contribution to separation of the taxa is also given in Table 1. Mean values for each character are given in Table 2.

Posterior cranial crest width, tarsus length, parotoid separation, and cranial crest length make relatively large contributions (in descending order) to the separation between taxa.

High values for nostril separation, anterior cranial crest width, head width, parotoid separation, eyelid length and width of the outer metatarsal tubercle ("spade") seem typical of *B. a. hemiophrys*, and high values of posterior cranial crest width, cranial crest length, width of largest wart in a blotch, tarsus length, and width of inner metatarsal tubercle seem typical of *B. a. americanus*.

When average discriminant weights were applied to the entire male reference sample of both species (1644 specimens) to obtain a discriminant value for each specimen, a single specimen, a *B.a. hemiophrys* from Delta, Manitoba, was misclassified, due to an error in measurement which was discovered on re-examination of the specimen and corrected. Recalculation with this measurement corrected gave a discriminant score of −10.2223, well within other *B. a. hemiophrys* scores.

Table 2. Mean values for each character measured in reference samples of male *Bufo a. americanus* and *B. a. hemiophrys*.

	Bufo a. americanus		*Bufo a. hemiophrys*	
	Group I	*Group II*	*Group I*	*Group II*
Snout-vent length	62.159164	62.004028	56.797302	56.924149
Nostril separation	3.9171495	3.9309130	4.2321920	4.2279654
Cranial crest length (right)	12.622904	12.582693	10.848487	10.848342
Cranial crest length (left)	12.634803	12.616809	10.868276	10.879306
Nostril to parotoid (right)	12.759092	12.718702	11.580885	11.566053
Nostril to parotoid (left)	12.764392	12.719232	11.629278	11.593626
Cranial crest width (anterior)	4.9079227	4.9357510	4.9805956	4.9777222
Cranial crest width (posterior)	6.7011232	6.6863594	4.1363468	4.1223583
Eyelid length	7.5224867	7.4919243	7.0796156	7.0735712
Head width	17.055084	17.057892	16.364105	16.374908
Tympanum diameter	4.9554949	4.9359989	4.1847582	4.1713829
Parotoid length (right)	11.721546	11.619533	10.434548	10.459098
Parotoid length (left)	11.711373	11.650821	10.654340	10.659307
Parotoid width (right)	6.4105911	6.3711004	6.2896471	6.3260069
Parotoid width (left)	6.3710337	6.3008833	6.2554150	6.338944
Parotoid separation	7.7693291	7.8577881	9.3114061	9.2901602
Spot length (right)	8.2746706	8.1989489	9.3226452	9.0972290
Spot length (left)	8.0421515	7.8604441	9.1226511	9.0211334
Spot width (right)	4.3023443	4.2669897	4.4395208	4.4684544
Spot width (left)	4.3076839	4.2541599	4.4869576	4.4262648
Wart width (right)	2.7535648	2.7376938	1.9527435	1.9794445
Wart width (left)	2.7809954	2.7781324	1.9698620	1.9940720
No. warts per spot (right)	2.7038832	2.9176750	4.4498777	4.3585358
No. warts per spot (left)	2.6941738	2.6973362	4.2933979	4.2365847
Tibia length	23.107529	23.026627	20.834091	20.847198
Largest wart on tibia (right)	3.0771122	3.0401163	2.1119099	2.1279736
Largest wart on tibia (left)	3.1288061	3.074981	2.1693630	2.1618786
Tarsus length	15.663939	15.685769	13.216190	13.232688
"Spade" width	4.7040462	4.6698914	5.0246153	5.0030861
Inner metatarsal tubercle width	2.4746885	2.4432659	2.0818386	2.1018782
Mean discriminant score (centroid):	24.037781	24.683716	−29.128174	−24.89318
Mahalanobis generalized distance:	between Group I centroids: 53.165955			
	between Group II centroids: 49.578034			

Table 3. Discriminant function, three scores, one measurement and four selected ratios for comparison of reference samples (pooled by locality) of male toads used in analysis of *Bufo americanus hemiophrys* from 24 samples (819 males) from Wyoming (W), Alberta (A), Saskatchewan (S), Manitoba (M), and North Dakota (DU) and of *B. a. americanus* from 18 samples (825 males) from Minnesota (MU), Ontario (O), Québec (Q), Prince Edward Island (P), New Brunswick (B) and Nova Scotia (N). The localities are mapped in Figure 2 and are listed in Appendix I with catalogue numbers and the dates of collection. Characters used are shown in Figures 4 and 5 and described in the Materials and Methods section under *Morphological character set*. The mean ($\bar{x}$) for each sample is given with the variance (s^2) (discriminant function score) or ± one standard deviation (s) (all other columns).

Locality number	Sample size	Discriminant function score $\bar{x}$	s^2	Cranial crest (cc) score $\bar{x} \pm s$	Post-orbital score $\bar{x} \pm s$	Venter score $\bar{x} \pm s$	Snout-vent (sv) length $\bar{x} \pm s$	Tibia/sv length ratio $\bar{x} \pm s$	Spot/sv length ratio $\bar{x} \pm s$	cc/head widths ratio $\bar{x} \pm s$	Spade/tarsus width/length ratio $\bar{x} \pm s$
Bufo americanus hemiophrys											
1W	36	−24.2	27.1	0.7±0.5	0.8±0.6	5.0±0.0	53.6±2.4	0.371±0.013	0.161±0.031	0.256±0.023	0.382±0.024
1A	8	−23.9	13.8	0.3±0.5	0.9±0.6	5.0±0.0	60.3±2.0	0.369±0.010	0.157±0.030	0.260±0.020	0.397±0.011
2A	48	−23.7	35.9	0.6±0.5	0.4±0.5	5.0±0.3	60.5±3.4	0.368±0.012	0.169±0.029	0.246±0.026	0.364±0.021
3A	36	−23.3	36.6	0.8±0.4	1.5±0.6	5.0±0.0	57.6±3.2	0.365±0.011	0.175±0.026	0.269±0.053	0.374±0.029
4A	55	−27.8	31.4	0.2±0.4	0.5±0.5	5.0±0.2	57.9±2.6	0.361±0.008	0.168±0.020	0.247±0.019	0.377±0.021
5A	27	−23.4	23.5	0.7±0.5	0.6±0.6	5.0±0.0	54.8±4.3	0.361±0.012	0.161±0.024	0.271±0.027	0.358±0.026
6A	36	−30.1	31.5	0.6±0.5	0.9±0.6	5.0±0.0	70.8±3.0	0.381±0.011	0.162±0.028	0.267±0.025	0.396±0.022
7A	20	−29.6	22.0	0.6±0.5	0.1±0.2	5.2±0.4	62.1±4.1	0.366±0.010	0.159±0.024	0.262±0.056	0.402±0.024
8A	68	−30.3	24.5	0.9±0.4	0.7±0.6	5.2±0.3	65.1±4.3	0.374±0.009	0.161±0.029	0.240±0.021	0.377±0.032
1S	18	−25.7	27.1	0.8±0.2	0.9±0.6	5.0±0.0	55.3±2.6	0.365±0.014	0.158±0.023	0.251±0.021	0.376±0.025
2S	40	−28.8	27.1	0.2±0.4	0.7±0.6	4.6±0.8	56.2±2.1	0.353±0.008	0.158±0.027	0.258±0.022	0.400±0.026
3S	88	−30.2	21.7	0.7±0.4	0.3±0.5	4.9±0.9	49.5±3.4	0.373±0.017	0.164±0.024	0.230±0.023	0.381±0.026
4S	22	−29.8	27.8	0.9±0.3	0.5±0.6	4.9±0.5	52.6±3.2	0.361±0.018	0.152±0.024	0.235±0.017	0.389±0.020
5S	22	−29.5	21.7	0.1±0.4	0.5±0.5	5.0±0.0	63.3±3.0	0.361±0.011	0.150±0.034	0.268±0.016	0.388±0.031
6S	36	−26.9	25.2	0.9±0.2	1.3±0.7	4.8±0.6	63.0±3.2	0.376±0.010	0.153±0.031	0.254±0.017	0.371±0.024
7S	27	−27.2	33.5	0.6±0.5	0.8±0.6	4.9±0.5	52.9±3.1	0.373±0.011	0.164±0.042	0.248±0.023	0.380±0.026
8S	24	−30.8	31.0	0.6±0.5	0.8±0.5	4.8±0.6	62.5±2.4	0.377±0.010	0.158±0.030	0.254±0.021	0.385±0.032
9S	74	−26.9	28.8	0.1±0.3	0.1±0.3	4.9±0.5	53.9±3.3	0.363±0.010	0.162±0.031	0.247±0.030	0.369±0.021
1M	14	−22.6	26.2	0.9±0.3	1.1±0.6	5.0±0.0	57.3±4.4	0.362±0.009	0.161±0.032	0.245±0.024	0.352±0.021
2M	20	−21.1	27.6	1.0±0.2	1.0±0.7	5.0±0.2	52.4±1.9	0.365±0.010	0.163±0.038	0.250±0.021	0.374±0.025
3M	8	−26.7	18.8	1.0±0.0	0.8±0.7	5.0±0.5	55.8±3.0	0.349±0.008	0.164±0.030	0.282±0.029	0.398±0.016
4M	49	−18.7	15.0	0.8±0.4	0.9±0.4	4.3±1.1	45.8±3.0	0.352±0.009	0.156±0.028	0.281±0.020	0.373±0.028
5M	34	−23.1	28.1	0.8±0.4	0.9±0.6	4.6±0.9	57.4±3.5	0.354±0.010	0.148±0.028	0.268±0.018	0.398±0.020
1DU	9	−21.9	45.9	1.0±0.0	1.0±0.9	5.0±0.0	52.2±4.2	0.373±0.008	0.152±0.027	0.256±0.012	0.377±0.013

Locality number	Sample size	Discriminant function score $\bar{x}$	s^2	Cranial crest (cc) score $\bar{x} \pm s$	Post-orbital score $\bar{x} \pm s$	Venter score $\bar{x} \pm s$	Snout-vent (sv) length $\bar{x} \pm s$	Tibia/sv length ratio $\bar{x} \pm s$	Spot/sv length ratio $\bar{x} \pm s$	cc/head widths ratio $\bar{x} \pm s$	Spade/tarsus width/length ratio $\bar{x} \pm s$
Bufo americanus americanus											
1MU	30	+17.5	44.0	3.0±0.0	2.0±0.2	4.9±1.0	60.6±4.8	0.367±0.012	0.108±0.040	0.380±0.026	0.306±0.015
1O	18	+23.5	49.5	2.9±0.3	1.8±0.4	5.8±0.5	60.5±5.1	0.378±0.013	0.215±0.130	0.379±0.027	0.305±0.020
2O	60	+16.3	28.9	3.0±0.0	2.0±0.0	5.7±0.5	52.0±4.8	0.372±0.012	0.154±0.056	0.370±0.025	0.299±0.027
3O	54	+13.3	38.0	3.0±0.0	1.5±0.6	4.8±1.0	58.4±4.6	0.352±0.045	0.114±0.045	0.339±0.031	0.298±0.016
4O	56	+24.5	58.7	3.0±0.0	1.9±0.3	5.1±0.6	64.7±4.5	0.372±0.014	0.128±0.045	0.395±0.028	0.305±0.023
5O	104	+26.3	38.4	3.0±0.0	1.9±0.2	5.0±0.6	64.5±4.7	0.363±0.016	0.128±0.045	0.390±0.020	0.299±0.025
6O	89	+26.0	29.7	3.0±0.0	1.9±0.4	4.5±1.2	60.7±3.2	0.380±0.011	0.134±0.043	0.393±0.022	0.289±0.024
7O	47	+33.0	47.1	3.0±0.0	1.9±0.4	4.2±1.0	65.1±6.9	0.385±0.012	0.089±0.029	0.408±0.021	0.283±0.021
8O	10	+35.3	45.0	3.0±0.0	2.0±0.0	4.4±1.1	67.7±7.3	0.382±0.011	0.093±0.023	0.402±0.015	0.288±0.026
1Q	14	+24.7	32.0	3.0±0.0	1.8±0.4	5.9±0.4	58.2±3.1	0.367±0.013	0.145±0.051	0.382±0.019	0.267±0.029
2Q	32	+33.1	52.2	3.0±0.0	2.0±0.0	5.9±0.3	64.9±3.9	0.351±0.009	0.147±0.068	0.431±0.029	0.307±0.014
3Q	38	+29.7	23.7	3.0±0.0	2.0±0.0	6.0±0.2	58.2±3.7	0.354±0.018	0.136±0.055	0.442±0.026	0.314±0.019
4Q	54	+24.8	42.0	3.0±0.0	2.0±0.1	4.7±1.1	66.5±4.0	0.375±0.014	0.140±0.043	0.379±0.025	0.295±0.017
5Q	22	+28.0	49.4	3.0±0.0	2.0±0.0	5.7±0.6	71.8±2.5	0.378±0.014	0.216±0.113	0.388±0.024	0.297±0.015
1P	49	+29.1	28.3	3.0±0.0	2.0±0.0	5.6±0.6	64.7±4.6	0.385±0.012	0.119±0.033	0.404±0.021	0.311±0.021
1B	53	+22.6	38.0	3.0±0.0	2.0±0.2	5.2±0.9	57.3±3.5	0.375±0.017	0.105±0.026	0.398±0.021	0.314±0.021
1N	55	+26.1	38.1	3.0±0.0	2.0±0.2	5.2±0.8	63.2±2.9	0.384±0.011	0.135±0.039	0.400±0.024	0.298±0.019
2N	40	+22.8	25.4	3.0±0.0	1.9±0.3	5.2±0.9	65.4±3.9	0.368±0.014	0.110±0.034	0.403±0.020	0.307±0.032

The sample size for each population sample of males is given in Table 3. Histograms for the pooled male reference samples are given in Figure 9.

The population mean for the discriminant score, ranging from −18.7 at Delta, Manitoba, to −30.8 at Qu'Appelle Valley in Saskatchewan for *B. a. hemiophrys* and +13.3 at Oxdrift, Ontario, to +33.8 at Rondeau Provincial Park, Ontario, for *B. a. americanus* (see Table 3). The highest values for *B. a. hemiophrys* are in the Aspen Parkland and Boreal Forest of Manitoba and adjacent North Dakota with slightly lower values across middle Saskatchewan and Alberta and northern Alberta, largely in the Boreal Forest. The mean of the disjunct Wyoming population falls with these. Southern Saskatchewan and Alberta populations have the lowest values from shortgrass prairie and Aspen Parkland habitats. The lowest values for *B. a. americanus* are from northern Minnesota (Hibbing), northwestern Ontario (Oxdrift) and the southern end of James Bay (Whitetop Creek).

The variance, as an indication of the dispersion of discriminant values within the population samples, is given in Table 3. Variance of *B. a. hemiophrys* populations were generally lower than for *B. a. americanus*, although higher variances are seen in *B. a. hemiophrys* populations in areas adjacent to eastern Manitoba.

Histograms of discriminant scores for each population sample, or pooled sample, are given in Figures 10 to 15. In general, they confirm that the values are roughly normally distributed within each population and that individual values are well away from the mid-point value of the discrimination axis. The closest approach to the mid-point is made by an individual *B. a. hemiophrys* from Perryvale, Alberta (Figure 10), and an *B. a. americanus* from Oxdrift, Ontario (Figure 14).

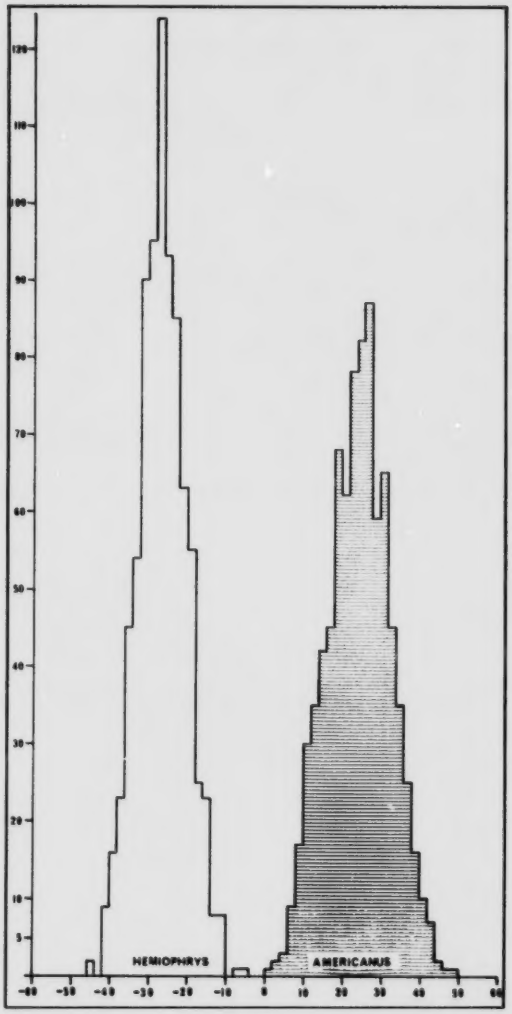


Figure 9. Histograms of individual discriminant scores for male reference samples: 819 *B. a. hemiophrys* (open) and 825 *B. a. americanus* (hatched). Vertical scale represents number of specimens, horizontal scale the discriminant score.

b. Univariate and Ratio Analysis of Males

In addition to the discriminant function analysis, the geographic variation in three scored characters: cranial crest, post-orbital crest and venter (Table 3), all excluded from the discriminant analysis, was examined. Snout-vent length and ratios of tibia/snout-vent, spot length/snout-vent, cranial crest posterior width/head width and outer metatarsal tubercle width/tarsus length (Table 3), were also examined for patterns of variation. These latter measurements had been used individually in the discriminant analysis.

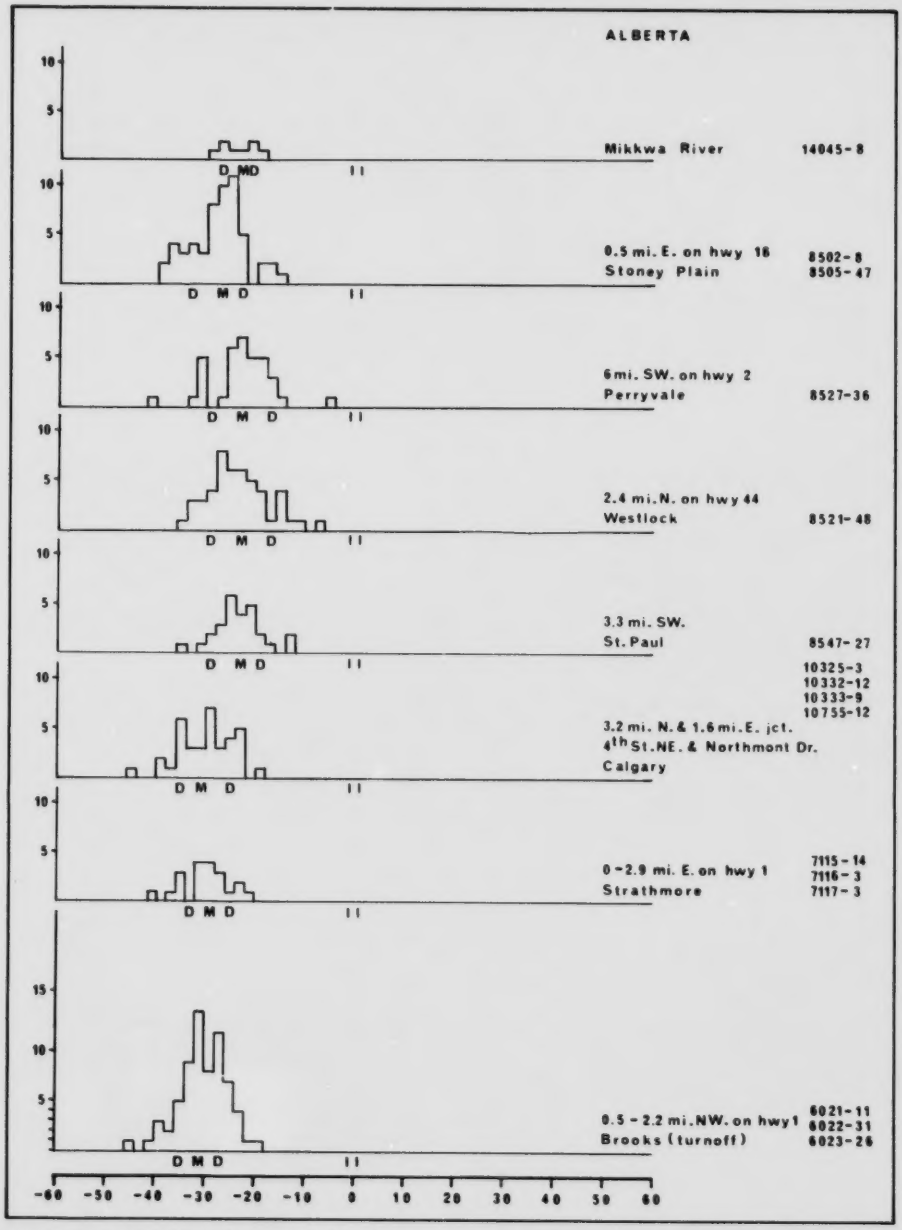


Figure 10. Histograms of individual discriminant function scores for eight Alberta reference collections of male *B. a. hemiophrys*. The two vertical lines indicate the mid-point of the discriminant analysis between *B. a. americanus* and *B. a. hemiophrys*. M indicates the position of the mean for the sample and D is the position of one standard deviation on either side of the mean (the unequal distances apparent in some histograms are due to rounding values to fit the whole number scale units used). For each sample the locality designation is given, and to the right of it the catalogue number (National Museums of Canada unless otherwise indicated) followed by a dash and the number of specimens in the sample.

The horizontal scale gives the discriminant score values, the vertical scale the number of individuals.

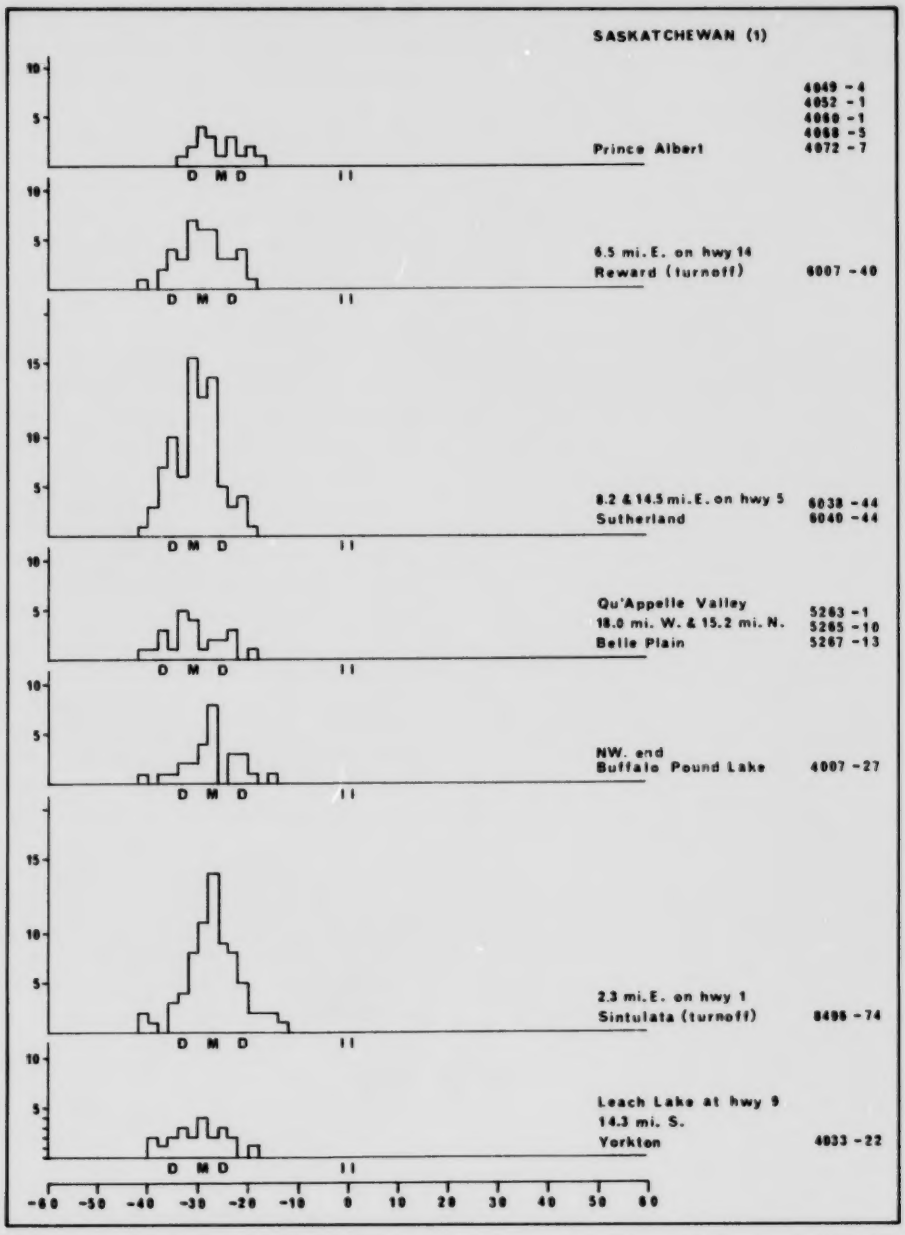


Figure 11. Histograms of individual discriminant function scores for seven Saskatchewan reference collections of male *B. a. hemiophrys*. See Figure 10 for explanation of letters, scale, etc.

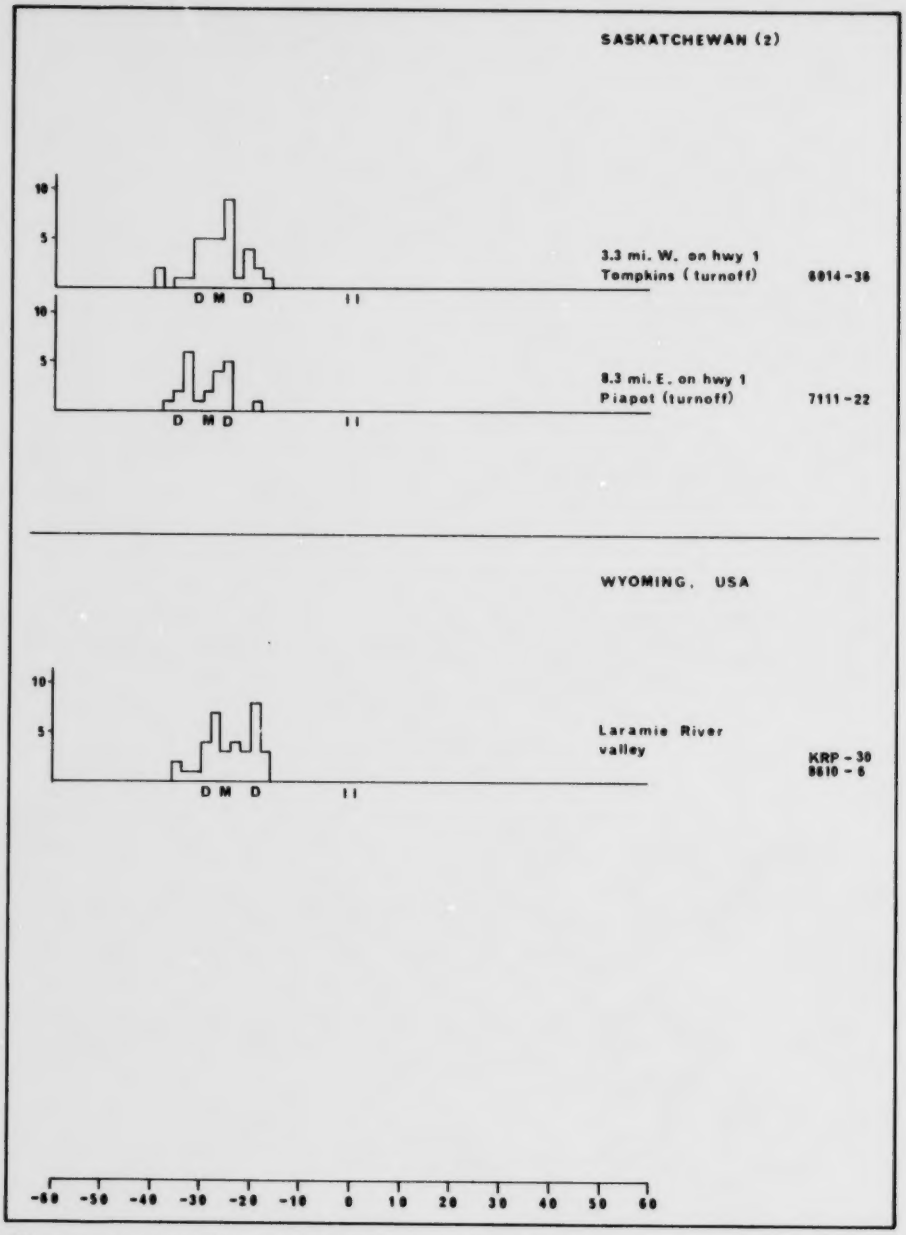


Figure 12. Histograms of individual discriminant function scores for two Saskatchewan and one Wyoming reference collections of male *B. a. hemiophrys*. See Figure 10 for explanation of letters, scale, etc.

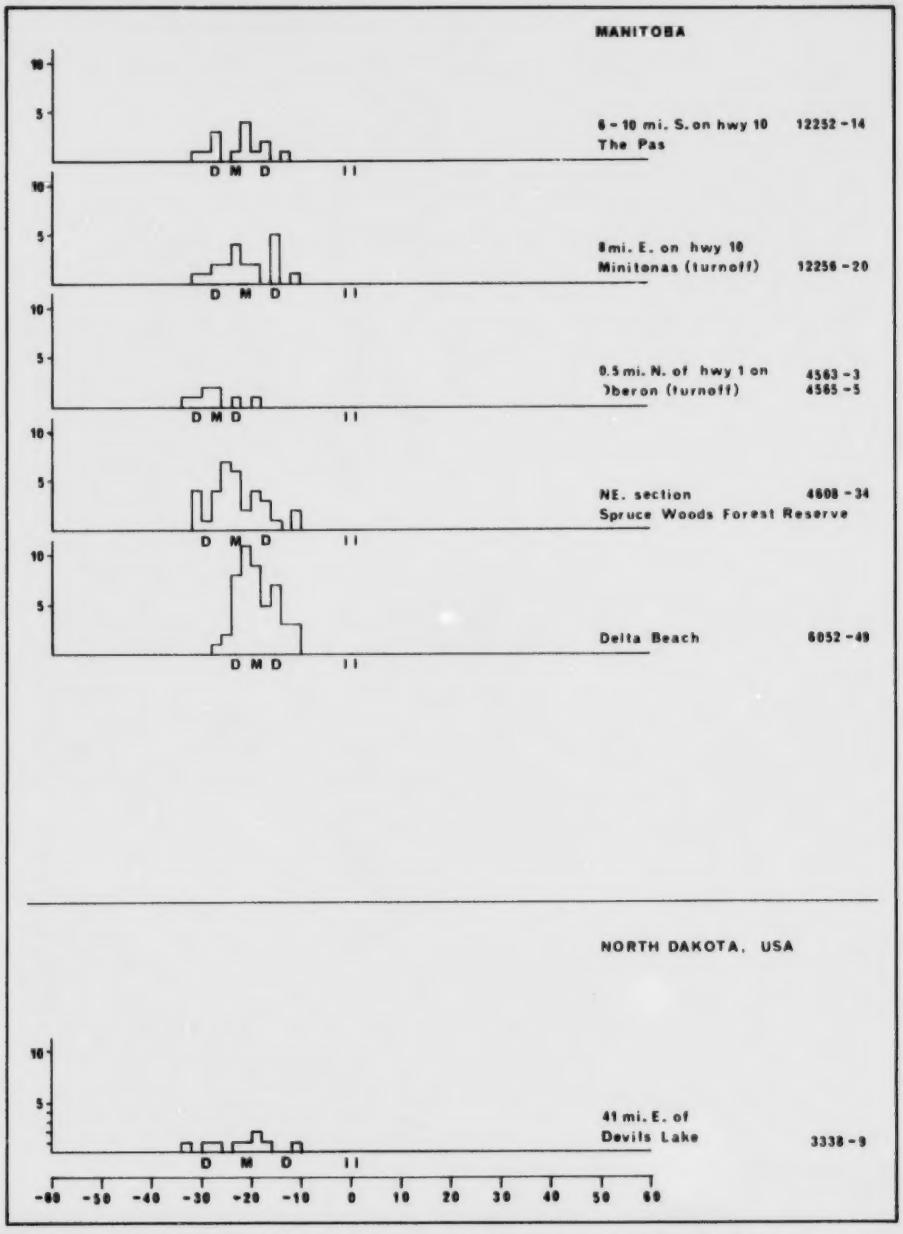


Figure 13. Histograms of individual discriminant function scores for five western and central Manitoba, and one North Dakota, reference collections of male *B. a. hemiophrys*. See Figure 10 for explanation of letters, scale, etc.

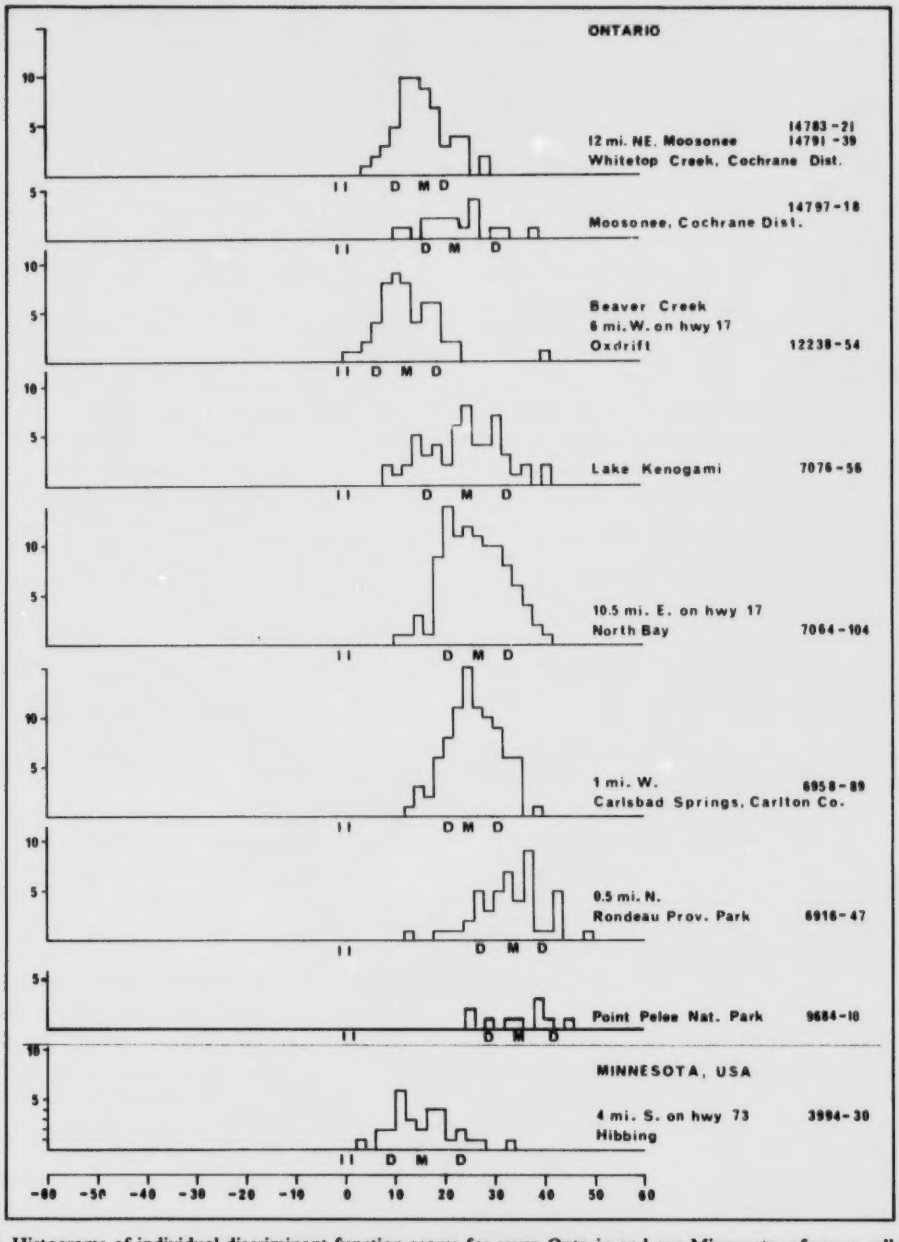


Figure 14. Histograms of individual discriminant function scores for seven Ontario and one Minnesota reference collections of male *B. a. americanus*. See Figure 10 for explanation of letters, scale, etc.

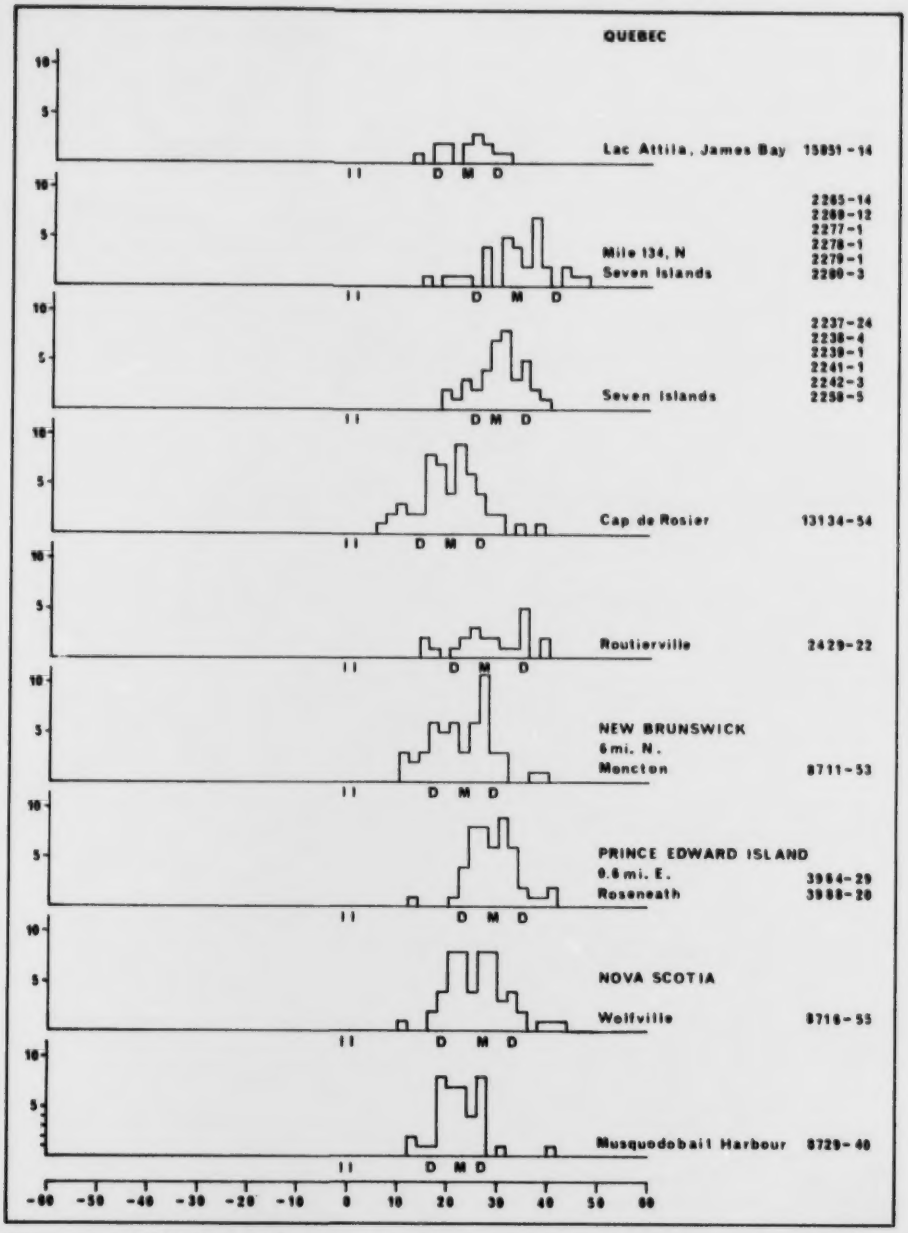


Figure 15. Histograms of individual discriminant function scores for five Quebec, one New Brunswick, one Prince Edward Island and two Nova Scotia reference collections of male *B. americanus*. See Figure 10 for explanation of letters, scale, etc.

Cranial Crest Score: Scores of 0 (boss) and 1 (grooved crest bridged posteriorly) were thought to be characteristic of *B. a. hemiophrys*. A score of 3 denoted a typical *B. a. americanus* crest. A score of 2 was assigned to any intermediate condition. Mean values for *B. a. hemiophrys* range from 0.1 to 1.0 and all mean values for *B. a. americanus* are 3.0. Values for *B. a. hemiophrys*, from Manitoba and North Dakota samples are consistently high, 1.0 to 0.8.

Post-orbital Crest Score: All *B. a. americanus* samples examined in this study had mean scores of 2.0 and 1.9 with the exception of 1.8 scores for the Lac Attila and Moosonee samples and 1.5 for Oxdrift. The means for *B. a. hemiophrys* ranged from 1.5 to 0.1 with high values (1.0 to 0.8) in eastern Manitoba, but considerable variation exists between populations elsewhere in the range, with one central Alberta population (Perryvale) scoring 1.5, and one southern Saskatchewan population (Tompkins) scoring 1.3.

Venter Score: This score ranged from 0 (immaculate venter) to 6 (reticulated venter) and as such covers the variation in *B. americanus* group toads. Means for *B. a. hemiophrys* varied from 4.3 to 5.0, with low values in central Manitoba (4.3 at Delta, and 4.6 at Spruce Woods). The highest values were in southern Alberta (5.2 at Brooks and at Sutherland) with northern values in Alberta, Saskatchewan and Manitoba all at 5.0. The range in *B. a. americanus* was greater, from 4.2 at Rondeau to 6.0 at Sept Îles. Northern *B. a. americanus* all tended to have high mean scores (above 5.5) and southern had the lowest scores (4.8 and lower), with those intermediate geographically tending toward intermediate scores (5.0–5.2). An anomaly is the Cap-des-Rosiers population at the tip of the Gaspé with a mean of 4.7. Less surprising is the 5.6 score for Roseneath, Prince Edward Island, putting it closer to northern populations in this character.

Snout-vent Length: Although there is an obvious difference in means between species (Table 2) for the whole reference sample there is a great deal of variation between population means within each species. For *B. a. americanus* the range was from 71.8 mm (Rothierville, Quebec) to 53.9 mm (Moosonee, Ontario). *B. a. hemiophrys* ranged from 70.8 mm (Calgary, Alberta) to 45.8 mm (Delta, Manitoba). For individual males, *B. a. hemiophrys* ranged from 79.1 mm (near Brooks, Alberta) to 41.3 mm (Delta, Manitoba) and *B. a. americanus* from 88.4 mm (near Rondeau Provincial Park, Ontario) to 42.1 mm (Whitetop Creek, near Moosonee, Ontario). Commonly, there is about a 20 mm spread from the largest to the smallest mature male in any given sample, though it ranges from about 5 to 25 mm, with the smaller *B. a. hemiophrys* generally having less of a spread (often 10–15 mm) within one population sample. However, in the Rondeau *B. a. americanus* sample the range is 38.4 mm.

The largest *B. a. hemiophrys* sample means (61.2 mm and larger) are in the prairie region of southern Alberta and Saskatchewan. There is no clear-cut geographic or vegetation region arrangement of the other groups, though there is a tendency for the sample means to be smaller in the east and larger in the west. Within *B. a. americanus* the smallest means are generally in the north (52.9 to 60.5 mm) but the large value of the sample from Mile 134 [Kilometre 215.7], north of Sept Îles, is out of line at 64.9 mm, and the small value from Moncton, New Brunswick, at 57.3 mm is aligned with the smaller northern means.

Tibia/Snout-vent Length: The relative length of the tibia is variable in both *B. a. americanus* and *B. a. hemiophrys*. Within *B. a. hemiophrys* there is little apparent pattern, though southern Manitoba means are among the lowest, and southern Alberta and Saskatchewan are among the highest. In *B. a. americanus* there is a tendency for northern scores to be low and southern means to be high.

Spot Length/Snout-vent Length: The range in *B. a. hemiophrys* (0.148 to 0.175) is much narrower than that in *B. a. americanus* (0.090 to 0.216). Within *B. a. hemiophrys* there is no obvious geographic pattern to the sample means of this ratio from north to south or between vegetation areas. In *B. a. americanus*, however, the northern populations (Moosonee, Lac Attila, Mile 134 [Kilometre 215.7], Sept Îles, Routhierville and Cap-des-Rosiers) have generally higher values (between 0.136 and 0.216) which encompass the *B. a. hemiophrys* means, while more southern populations (Oxdrift, Hibbing, Rondeau, Moncton, Roseneath and Musquodoboit) have low values (0.119–0.090). Kenogami, North Bay and Ottawa are intermediate (0.128 to 0.134), as is Wolfville (0.132). That the western *B. a.*

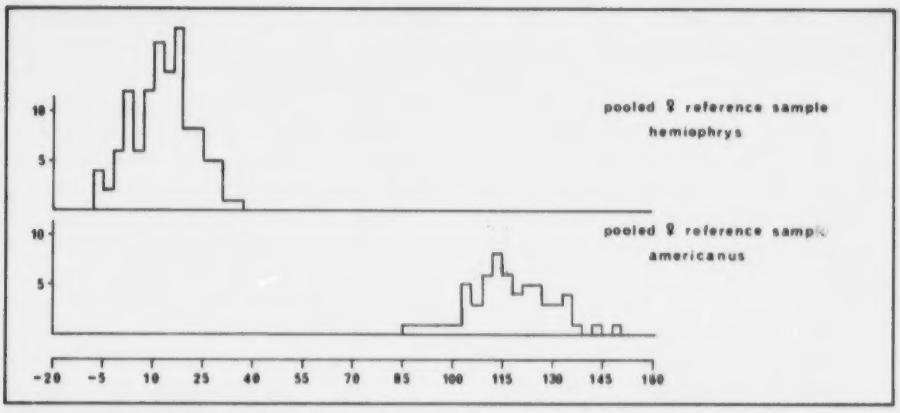


Figure 16. Histograms of individual discriminant function scores for pooled reference samples of females of *B. a. hemiophrys* and *B. a. americanus*. Vertical axis shows number of individuals and the horizontal gives the score. List of localities, dates, catalogue numbers and number of specimens is given in Appendix I.

americanus have smaller dorsal spots than *B. a. hemiophrys* heightens the contrast between the taxa in adjacent western Ontario and eastern Manitoba.

Posterior Width of the Cranial Crest/Head Width: *B. a. hemiophrys* population means of this character range from 0.240 to 0.281. There does not appear to be any obvious geographic pattern to the ratio within the taxa, as both high and low means occur generally throughout the range, except that the two highest values (0.280, 0.281) are in central Manitoba whereas all other values are 0.269 or below.

In *B. a. americanus* the values for means vary from 0.339 to 0.442. The low value is from Oxdrift in western Ontario. The next lowest value is 0.370 from Whitetop Creek. All other values are 0.379 (Cap-des-Rosiers, Moosonee) or above. Values at 0.398 or above are predominant in the east (six out of eight means). In extreme southern Ontario values are 0.408 at Rondeau and 0.401 at Point Pelee. The other central values are 0.390–0.395 in the Kenogami to Ottawa area and 0.382 at Lac Attila. There is no clear north-south cline.

Spade/Tarsus: The mean values for *B. a. hemiophrys* populations varied from 0.352 to 0.402. The lowest is from The Pas, Manitoba, and the highest is from Strathmore in western Alberta. In *B. a. americanus* means varied from 0.267 at Lac Attila to 0.314 at Moncton, New Brunswick, and Sept Îles, Quebec. No clear geographic trends are evident in either taxon.

c. Discriminant Function Analysis of Females

The discriminant weights and means for each character for females are given in Table 4.

Posterior cranial crest width made the largest contribution as it did in the male analysis. Tarsus length and parotoid separation also make contributions but the largest wart on the right tibia, the cranial crest length (right side) and distance from nostril to parotoid on the left side seem to make disproportionate contributions to this separation compared to their contribution in the discriminant analysis of males. The Mahalanobis generalized distance is 104.6573390 for female reference samples compared to less than half that value (51.3517512; Table 1) in the male analysis.

The histograms of the individual scores for the female reference sample are plotted in Figure 16. Because of the generally higher weights in the discrimination and larger values of individual measurements the scores are higher than obtained in the male discrimination. As individual population samples were small, these have not been plotted separately.

3. Morphological Character Comparison of Southeastern Manitoba *Bufo*.

a. Discriminant Scores of Males

Figure 3 gives the distribution of samples in eastern Manitoba. None of these collections were

Table 4. Discriminant weights and mean values for each character in discriminant analysis of female reference samples of *Bufo a. americanus* and *B. a. hemiophrys*, and the weight contribution of the Mahalanobis generalized distance.

	Discriminant Weights	Means *Bufo a. americanus*	Means *Bufo a. hemiophrys*	Contribution
Snout-vent length	+0.05570437	72.499863	59.72431	+0.711654
Nostril separation	−12.574883	4.5457535	4.3228445	−2.803055
Cranial crest length (right)	+8.7230959	14.881289	11.388057	+30.471798
Cranial crest length (left)	+0.99025917	14.857541	11.420262	+3.403797
Nostril to parotoid (right)	+2.4354296	15.081285	12.211794	+6.988443
Nostril to parotoid (left)	−0.99551696	15.127056	12.233808	−2.880277
Cranial crest width (anterior)	−5.6168242	5.8474321	5.2152014	−3.551129
Cranial crest width (posterior)	+11.437762	8.1440296	4.3813000	+43.037206
Eyelid length	−0.16800469	8.6168976	7.2346783	−0.232219
Head width	−0.66918558	20.243988	16.905833	−2.233842
Tympanum diameter	−0.59421539	5.7406559	4.3101177	−0.850048
Parotoid length (right)	+0.41642243	13.593149	10.921095	+1.112703
Parotoid length (left)	+1.6200924	13.721965	11.038900	+4.346813
Parotoid width (right)	−1.5974340	7.2253618	6.5355158	−1.101983
Parotoid width (left)	−3.1625957	7.1067219	6.5041580	−1.905666
Parotoid separation	−6.1137037	9.1507912	9.7041588	+3.383126
Spot length (right)	+0.13028830	10.289782	9.6245070	+0.086678
Spot length (left)	+0.23475748	10.540627	9.6906052	+0.199549
Spot width (right)	+4.4119072	5.2016764	4.5041809	+3.077285
Spot width (left)	−1.2844067	5.1830263	4.4414635	−0.952468
Wart width (right)	+5.1356106	3.2084694	2.0423670	+5.988648
Wart width (left)	+3.3310843	3.1898260	2.0398245	+3.830752
No. warts per spot (right)	+0.66464460	2.6440678	4.4491520	−1.199739
No. warts per spot (left)	−1.7433395	2.6779652	4.3135586	+2.851395
Tibia length	−1.012804	26.891434	20.971939	−5.995288
Largest wart on tibia (right)	+6.8065100	3.7305031	2.1245699	+10.930800
Largest wart on tibia (left)	+0.53891832	4.4559221	2.1906691	+1.220786
Tarsus length	+2.0391092	18.149078	13.522799	+9.433488
"Spade" width	−10.362802	5.6287861	5.3338470	−3.056395
Inner metatarsal tubercle width	+0.42757177	3.0406723	2.2347364	+0.344595
Mean discriminant score (centroid):		117.73267	13.075331	
Mahalonobis generalized distance	104.6573390			

included in the reference samples used for the discriminations between *B. a. hemiophrys* and *B. a. americanus*. These collections can be divided roughly into six west-east transects for discussion:

1. The northern transect: localities 1 to 14.
2. The Beausejour transect: localities 15 to 21.
3. The Vivian transect: localities 22 to 38.
4. The Trans-Canada Highway (Highway 1): localities 39 to 43.
5. The Marchand transect: localities 44 to 48.
6. The southern transect: localities 49 to 61.

Sample sizes are given in Table 5. There is great disparity in number (2–268 individuals) largely due to local weather conditions during the collecting period. Some localities represent pooled adjacent samples, several samples from separate visits during the same breeding season and/or several collections taken in different years (see Appendix I).

Table 5 gives the mean discriminant score for each population sample. That western scores are low and eastern ones high is immediately apparent. The rapid transition from minus to plus (between −10 and +10) scores occurs largely in the eastern half of the Manitoba Lowlands Forest Region (of Rowe 1959), with the southern transect transition in the western extreme of the Great Lakes Forest. Table 5 gives the variance values for these samples. Generally a zone of very high variances (in the 80–258 range) clearly coincides with the intermediate means in five of the

Table 5. Discriminant function, three scores, one measurement and four selected ratios for comparison of six transects totaling 61 samples (2460 males) of *Bufo* from southeastern Manitoba. The localities are mapped in Figure 3 and are listed in Appendix I with catalogue numbers and dates of collection. Characters used are shown in Figures 4 and 5 and described in the Material and Methods section under *Morphological character set*. The mean, $\bar{x}$, for each sample is given with the variance, s^2 (discriminant function score), or ± one standard deviation, s (all other columns).

Locality number	Sample size n	Discriminant function score $\bar{x}$	s^2	Cranial crest (cc) score $\bar{x} \pm s$	Post-orbital score $\bar{x} \pm s$	Venter score $\bar{x} \pm s$	Snout-vent length $\bar{x} \pm s$	Tibia/sv length ratio $\bar{x} \pm s$	Spot/sv length ratio $\bar{x} \pm s$	cc/head widths ratio $\bar{x} \pm s$	Spade/tarsus width/length ratio $\bar{x} \pm s$
Northern transect											
1	23	−20.9	51.6	1.0±0.3	1.0±0.6	4.4±1.0	59.5±4.0	0.359±0.009	0.172±0.034	0.283±0.027	0.361±0.024
2	15	−18.0	41.6	1.0±0.4	0.7±0.6	4.0±1.3	54.8±2.4	0.348±0.086	0.165±0.029	0.271±0.021	0.388±0.093
3	13	−13.6	23.0	1.2±0.4	1.2±0.7	5.1±0.3	59.2±4.8	0.364±0.011	0.167±0.031	0.283±0.032	0.346±0.016
4	15	−12.4	62.9	1.9±0.5	1.1±0.6	4.1±1.1	58.6±3.7	0.365±0.010	0.170±0.038	0.297±0.042	0.365±0.040
5	31	−08.9	61.2	1.7±0.6	1.7±0.5	4.4±0.9	62.6±4.3	0.368±0.010	0.143±0.035	0.290±0.036	0.343±0.022
6	30	−07.3	47.3	1.5±0.8	1.4±0.8	4.0±0.5	59.2±4.0	0.370±0.014	0.135±0.030	0.279±0.034	0.340±0.030
7	42	−14.0	38.2	1.2±0.4	0.6±0.5	3.9±1.2	53.8±3.2	0.366±0.012	0.141±0.027	0.274±0.026	0.341±0.028
8	24	−09.4	23.8	1.6±0.5	1.0±0.6	4.6±1.3	54.1±4.2	0.362±0.011	0.134±0.022	0.290±0.028	0.352±0.017
9	18	−10.1	30.6	1.1±0.3	1.3±0.5	4.6±0.8	57.7±2.7	0.368±0.012	0.160±0.041	0.294±0.033	0.323±0.055
10	31	+07.2	43.8	2.9±0.3	1.8±0.4	4.7±0.9	61.9±3.3	0.354±0.013	0.100±0.033	0.346±0.032	0.326±0.018
11	12	+10.3	64.2	2.9±0.3	1.7±0.7	4.7±0.9	62.3±5.3	0.364±0.012	0.144±0.075	0.367±0.041	0.333±0.018
12	15	+11.8	49.4	3.0±0.0	1.8±0.4	4.7±1.0	60.5±6.1	0.360±0.011	0.118±0.039	0.360±0.028	0.310±0.021
13	3	+05.8	29.6	3.0±0.0	2.0±0.0	5.0±0.0	64.0±1.9	0.362±0.006	0.110±0.051	0.338±0.008	0.323±0.038
14	2	+18.5	03.5	3.0±0.0	2.0±0.0	5.0±0.0	68.3±0.8	0.357±0.011	0.102±0.028	0.381±0.001	0.286±0.028
Beausejour transect											
15	13	−17.8	23.4	1.0±0.0	1.3±0.6	4.4±1.0	52.2±1.7	0.356±0.008	0.167±0.023	0.285±0.018	0.386±0.020
16	21	−15.1	20.2	0.9±0.4	1.1±0.5	4.0±1.3	56.4±2.9	0.363±0.010	0.160±0.035	0.291±0.028	0.366±0.019
17	70	−10.7	45.1	1.2±0.7	1.2±0.6	4.1±1.0	56.5±2.8	0.365±0.012	0.164±0.032	0.281±0.029	0.343±0.023
18	16	−07.0	94.0	1.6±0.7	1.1±0.5	4.1±1.2	59.7±2.9	0.364±0.011	0.171±0.033	0.290±0.036	0.346±0.024
19	7	+16.0	133.5	2.9±0.4	1.9±0.4	5.1±0.4	63.6±4.7	0.368±0.010	0.121±0.040	0.383±0.026	0.308±0.033
20	48	+15.3	42.6	3.0±0.0	2.0±0.2	5.1±0.6	63.1±3.3	0.373±0.017	0.141±0.046	0.375±0.028	0.316±0.019
21	13	+13.6	54.2	3.0±0.0	1.8±0.4	5.1±0.9	61.0±3.2	0.369±0.014	0.123±0.035	0.366±0.038	0.316±0.021
Vivian transect											
22	38	−07.0	86.6	1.7±0.7	1.6±0.5	4.6±0.9	60.1±5.1	0.362±0.013	0.156±0.037	0.305±0.046	0.345±0.026
23	29	+11.3	110.3	2.9±0.3	1.9±0.4	4.7±0.9	63.7±6.4	0.362±0.011	0.128±0.043	0.355±0.041	0.321±0.024
24	56	+10.3	50.4	3.0±0.0	1.9±0.3	5.0±1.0	62.5±5.1	0.370±0.012	0.119±0.034	0.360±0.028	0.323±0.019
25	49	+11.2	26.3	3.0±0.1	1.8±0.4	4.5±1.0	58.3±4.3	0.359±0.014	0.108±0.035	0.364±0.024	0.320±0.022
26	24	+13.6	33.9	3.0±0.0	1.8±0.4	5.2±0.5	60.7±5.2	0.350±0.010	0.126±0.044	0.369±0.037	0.324±0.022
27	34	+15.4	35.5	3.0±0.0	2.0±0.0	5.0±0.8	62.4±4.3	0.368±0.014	0.136±0.043	0.373±0.023	0.314±0.023
28	55	+13.3	37.9	3.0±0.2	1.9±0.3	5.0±0.9	60.7±5.4	0.374±0.011	0.134±0.046	0.364±0.024	0.319±0.038

Locality number	Sample size n	Discriminant function score $\bar{x}$	s^2	Cranial crest (cc) score $\bar{x} \pm s$	Post-orbital score $\bar{x} \pm s$	Venter score $\bar{x} \pm s$	Snout-vent length $\bar{x} \pm s$	Tibia/sv length ratio $\bar{x} \pm s$	Spot/sv length ratio $\bar{x} \pm s$	cc/head widths ratio $\bar{x} \pm s$	Spade/tarsus width/length ratio $\bar{x} \pm s$
Trans-Canada highway transect											
29	37	−15.3	32.4	0.9±0.4	1.0±0.6	4.6±0.7	54.5±2.4	0.354±0.010	0.178±0.027	0.260±0.029	0.352±0.022
30	6	−19.2	80.0	0.8±0.4	0.7±0.5	4.2±1.0	56.1±1.3	0.355±0.010	0.155±0.028	0.297±0.044	0.380±0.020
31	143	−16.9	31.4	1.1±0.6	1.0±0.5	4.7±0.8	54.2±3.7	0.352±0.029	0.164±0.033	0.266±0.030	0.359±0.023
32	20	−13.7	30.2	1.3±0.4	1.1±0.6	4.4±1.1	57.1±3.5	0.352±0.023	0.170±0.022	0.288±0.033	0.353±0.023
33	46	−13.8	45.1	1.2±0.5	1.1±0.5	4.9±0.5	54.0±3.7	0.334±0.014	0.164±0.032	0.291±0.038	0.366±0.022
34	37	+01.8	97.8	2.5±0.8	1.8±0.4	4.8±0.9	58.0±3.8	0.360±0.018	0.145±0.038	0.331±0.044	0.330±0.041
35	268*	+05.4	89.5	2.5±0.6	1.8±0.4	4.7±0.8	59.0±4.2	0.357±0.014	0.140±0.045	0.348±0.036	0.320±0.033
36	24	+12.9	18.4	3.0±0.0	1.9±0.3	5.1±0.8	62.8±3.1	0.352±0.010	0.123±0.048	0.367±0.027	0.306±0.018
37	9	+10.1	33.8	3.0±0.0	1.9±0.3	5.3±0.5	61.6±4.0	0.350±0.007	0.115±0.041	0.365±0.040	0.326±0.019
38	4	+09.7	223.1	3.0±0.0	1.8±0.5	5.5±0.6	59.8±1.7	0.364±0.002	0.163±0.023	0.369±0.040	0.308±0.016
39	174	+14.2	28.6	3.0±0.2	2.0±0.1	4.9±0.7	60.3±3.9	0.356±0.011	0.117±0.039	0.379±0.025	0.311±0.023
40	35	+10.8	34.3	3.0±0.0	1.9±0.3	4.9±0.9	62.6±3.4	0.355±0.015	0.119±0.034	0.360±0.031	0.314±0.023
41	7	+18.6	47.7	3.0±0.0	2.0±0.0	5.1±0.7	63.7±2.6	0.366±0.013	0.121±0.047	0.373±0.029	0.303±0.019
42	7	+13.8	30.0	3.0±0.0	1.9±0.4	5.4±0.5	64.6±4.7	0.362±0.011	0.115±0.043	0.383±0.035	0.328±0.011
43	146	+14.6	34.8	3.0±0.0	1.9±0.3	4.9±0.6	59.5±3.7	0.355±0.011	0.121±0.049	0.379±0.063	0.314±0.023
Marchand transect											
44	86	−14.4	27.7	0.8±0.4	1.2±0.6	4.2±1.0	45.6±2.9	0.352±0.011	0.170±0.035	0.276±0.024	0.344±0.026
45	6	−14.8	42.2	1.2±0.4	1.7±0.5	5.2±0.4	62.3±3.0	0.362±0.007	0.186±0.016	0.299±0.043	0.374±0.022
46	25	+10.1	135.2	2.7±0.5	2.0±0.0	4.9±0.8	66.3±3.8	0.365±0.013	0.140±0.032	0.367±0.043	0.328±0.031
47	71	+17.7	38.9	3.0±0.0	2.0±0.3	4.9±0.5	59.8±3.1	0.361±0.012	0.115±0.047	0.384±0.030	0.310±0.029
48	46	+16.7	35.4	3.0±0.0	2.0±0.2	4.8±0.9	61.0±4.0	0.359±0.009	0.117±0.046	0.363±0.028	0.309±0.024
Southern transect											
49	44	−13.5	33.3	1.3±0.5	0.7±0.5	4.7±0.8	58.7±3.6	0.365±0.011	0.159±0.027	0.276±0.030	0.352±0.023
50	37	−14.4	17.0	0.8±0.4	1.0±0.7	4.6±1.0	43.5±2.4	0.346±0.012	0.167±0.030	0.273±0.029	0.371±0.033
51	55	−13.0	28.8	0.8±0.4	1.2±0.7	4.6±0.7	48.2±2.7	0.355±0.011	0.169±0.026	0.292±0.026	0.354±0.029
52	45	−11.8	34.9	1.5±0.5	1.5±0.6	4.4±1.0	55.2±3.3	0.364±0.012	0.173±0.035	0.292±0.028	0.349±0.023
53	9	−12.9	80.0	1.4±0.5	1.6±0.5	4.8±1.0	56.0±2.9	0.365±0.013	0.176±0.012	0.297±0.036	0.347±0.026
54	28	−04.3	101.1	2.0±0.8	1.5±0.5	4.4±1.2	60.3±4.9	0.369±0.012	0.142±0.038	0.313±0.035	0.345±0.046
55	14	−03.2	122.3	2.5±0.5	1.9±0.4	4.5±0.8	64.2±5.6	0.372±0.013	0.166±0.038	0.336±0.043	0.327±0.022
56	20	+12.5	258.1	2.6±0.8	2.0±0.2	4.3±1.1	67.1±4.2	0.379±0.011	0.125±0.035	0.345±0.039	0.301±0.028
57	67	−05.5	64.8	1.8±0.5	1.5±0.7	3.2±1.6	53.2±4.1	0.363±0.012	0.162±0.029	0.314±0.038	0.344±0.028
58	25	+14.7	102.1	2.9±0.3	1.9±0.3	4.8±1.0	60.2±4.0	0.370±0.018	0.125±0.038	0.372±0.030	0.305±0.041
59	35	+12.2	59.0	2.9±0.2	1.9±0.3	4.5±0.8	61.6±3.9	0.361±0.012	0.142±0.042	0.367±0.028	0.321±0.030
60	69	+15.7	28.8	3.0±0.0	1.9±0.4	4.5±1.0	60.2±3.7	0.361±0.011	0.115±0.034	0.377±0.083	0.297±0.020
61	68	+13.5	27.4	3.0±0.0	1.9±0.3	4.5±0.9	57.4±3.7	0.365±0.014	0.118±0.037	0.365±0.028	0.306±0.021

* Discriminant function based on 268 specimens, univariate and ratio statistics based on 214 specimens (see Appendix I).

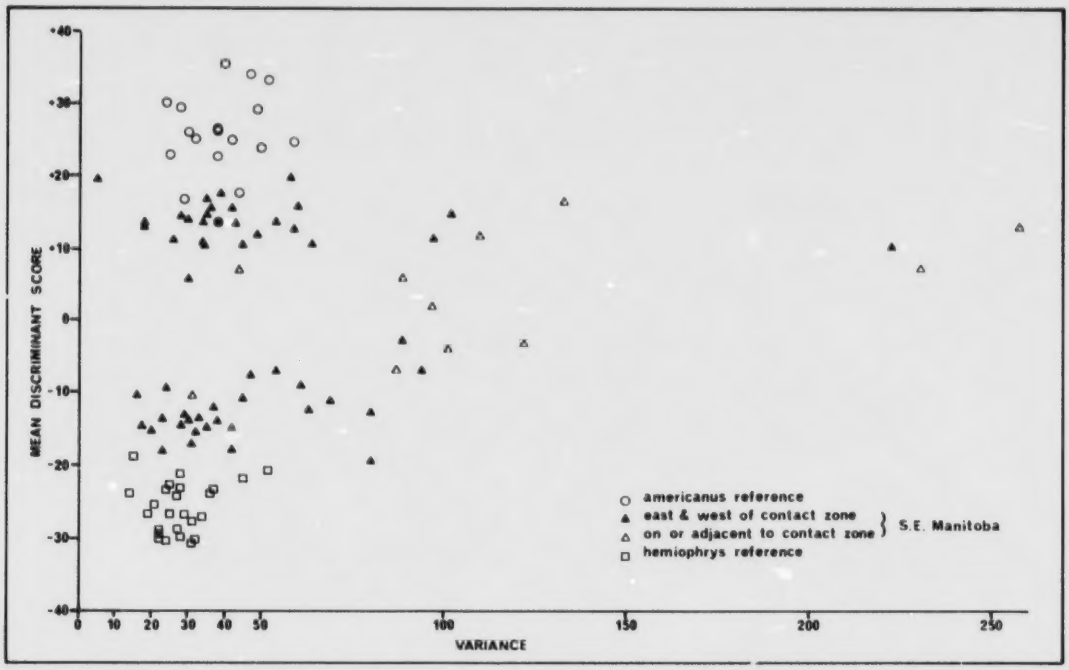


Figure 17. Relationship of mean discriminant score and variance for reference samples of *B. a. americanus* and *B. a. hemiophrys* and for *Bufo* samples from eastern Manitoba. The most intermediate scores have the largest variances.

transects. In the northern transect the variances are not as large in the transition zone but values of 61 and 64 are larger than those in eastern and western samples in the region. Perhaps the exact middle of the transition zone has not been sampled as effectively in this transect as it was in the others. That high variances are correlated with intermediate scores is further brought out by Figure 17 where mean discriminant scores are plotted against the variance. Most very high variances are from the contact zone.

Figures 18 to 24 present histograms of the scores of individuals within populations sampled. In the northern transect (Figure 18) the transition occurs between localities 9 and 10. Various other samples show occasional individuals over the mid-point of the discrimination axis. In the Beausejour transect (Figure 19) the shift occurs between locality 18 and locality 19, but localities 17 and 20 show slight extensions over the mid-point of the discrimination. In the Vivian transect (Figure 20) the shift is between localities 22 and 23 but the samples from locality 24 show some extensions over the mid-point. This is also marginally evident in locality 25 and also in locality 28. The individual left of mid-point in the latter sample may be simply a chance extreme recombination from introgression. In the Trans-Canada Highway transect, which is the most thoroughly covered of all the transects (Figures 21 and 22) the shift can be seen between locality 33 and 36. Localities 34 and 35 clearly are intermediate populations from the centre of the zone of contact. Locality 38 shows one individual to the left side of mid-point and this is either a stray from a western locality or a chance extreme recombination. The Marchand Transect (Figure 23) is the most poorly sampled but clearly locality 46 has an intermediate population. The southern transect (bottom of Figure 24 and Figure 25) cuts a diagonal through the intermediate zone and one sample (locality 57) is geographically "out of place" in the arrangement of these samples as it is south of the general sample line (see Figure 3). The number of intermediate populations is greater because of the NW–SE axis of sampling instead of the general west-east line in other transects except the northern one. Clearly intermediate populations lie at locality 54, locality 55 and locality 56. Locality 57 is south of these and is largely on the *B. a. hemiophrys* side of the axis

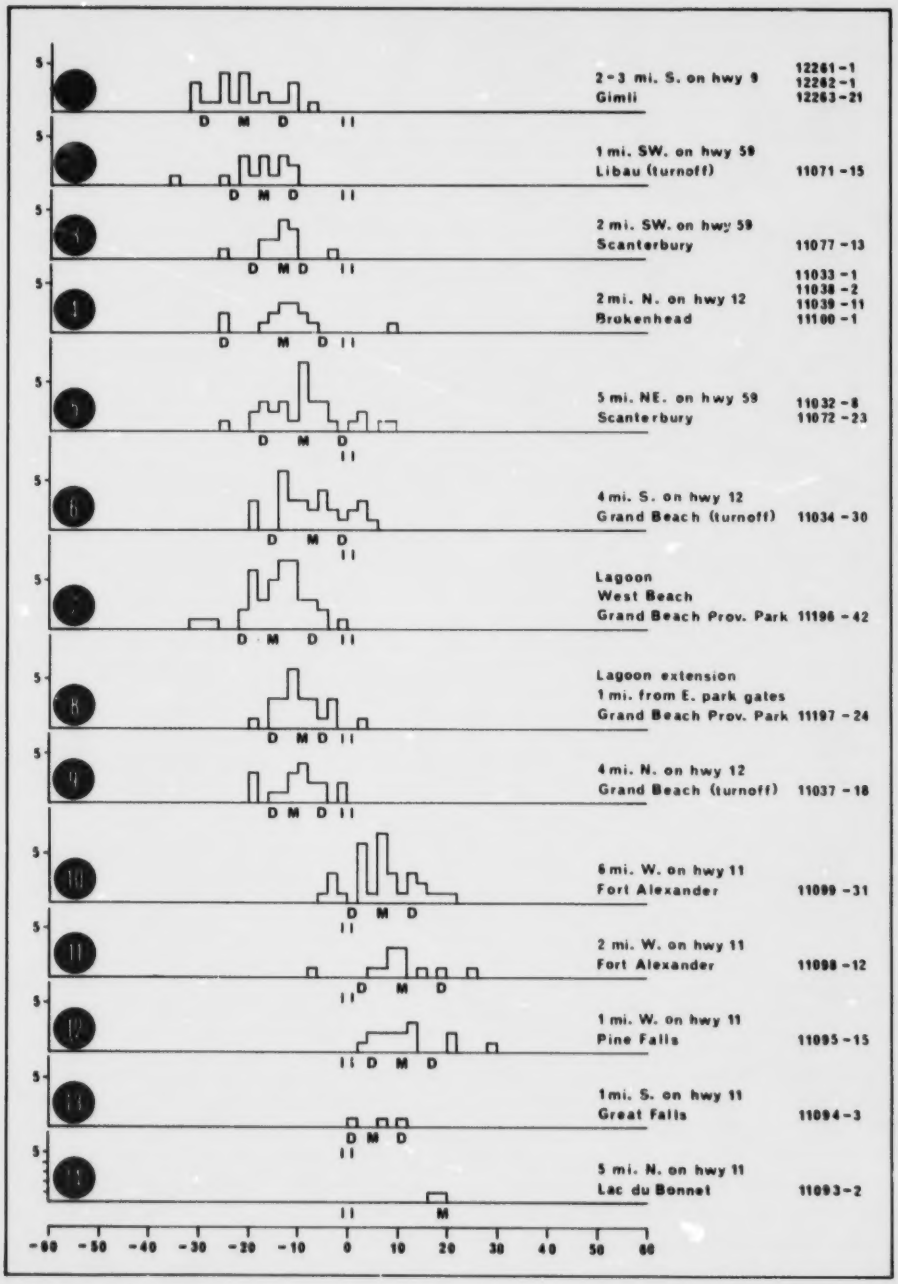


Figure 18. Histograms of individual discriminant function scores of collections of male *Bufo* for collecting stations 1–14 along the northern transect through southeastern Manitoba. The two vertical lines indicate the mid-point of the discriminant analysis between *B. a. americanus* and *B. a. hemiophrys* reference samples. See Figure 10 for explanation of letters, scale, etc.

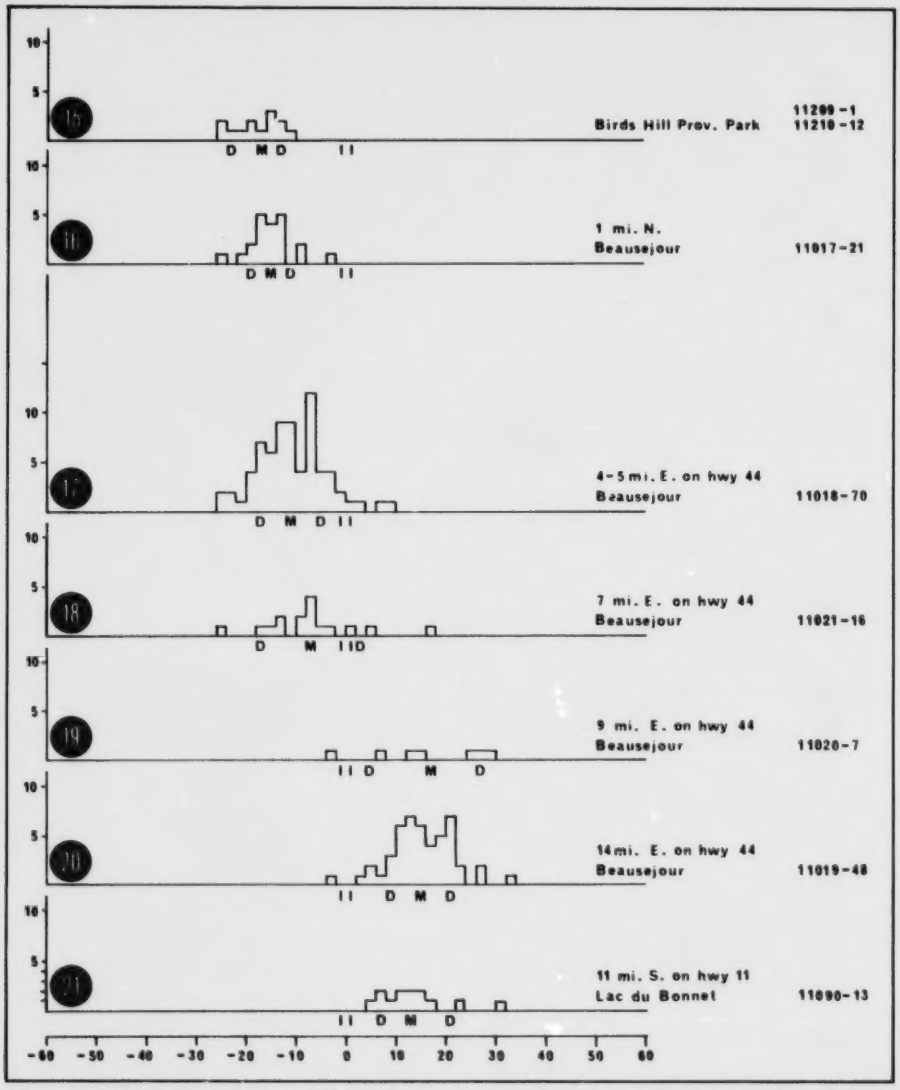


Figure 19. Histograms of individual discriminant function scores of collections of male *Bufo* for collecting stations 15-21 along the Beausejour transect through southeastern Manitoba. An explanation of letters, scale, etc. is given in Figure 10.

but has intermediate and *B. a. americanus*-like individuals. Other localities northwest and southeast show occasional individuals across the mid-point.

The mean discriminant scores for populations to the east and west of the narrow intermediate zone defined by the line connecting these points are consistently above 10 to the west with two exceptions, locality 13 mean +5.8 and locality 38 mean +9.8. In both these cases the sample size is very small, five and four specimens respectively, and only in the latter sample is an individual on the *B. a. hemiophrys* side of the discriminant axis present. However, the *B. a. hemiophrys* means in eastern Manitoba −10.2 to −19.2 are consistently higher than those for the reference sample (compare Table 3 and Table 5). All reference sample means, except Delta at −18.7, are below −20.0. Similarly all *B. a. americanus* means in eastern Manitoba (with the exception of localities 13 and 38 mentioned above) are +10.1 to +18.6. In the reference sample of *B. a. americanus*, means are all above +20.0 with the exception of Oxdrift (+13.3), Hibbing (+17.5), and Whitetop Creek, NE of Moosonee (+16.2).

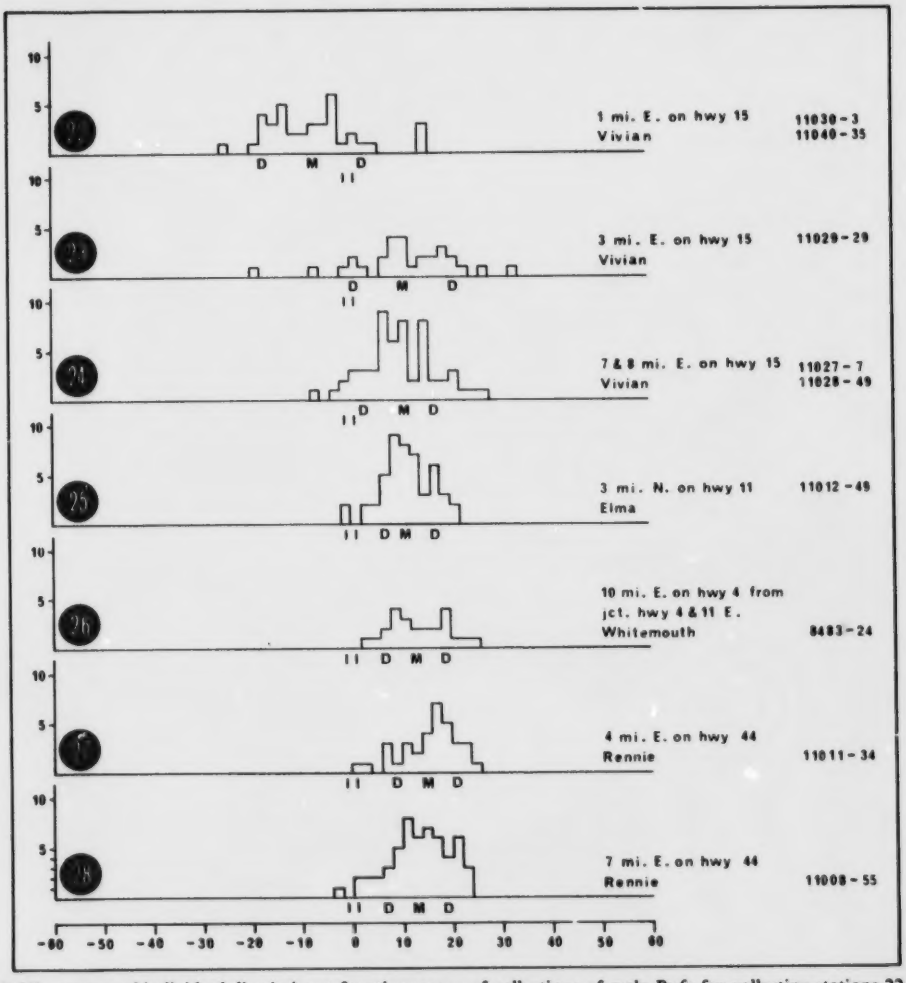


Figure 20. Histograms of individual discriminant function scores of collections of male *Bufo* for collecting stations 22–28 along the Vivian transect through southeastern Manitoba. An explanation of letters, scale, etc. is given in Figure 10.

Figures 25, 26 and 27 show histograms of individual discriminant scores by date of collection for localities 31, 35, and 39, respectively. The pooled results of these scores were used in Figures 21 and 22 as population histograms and in Table 5 for means and variances.

For locality 31, a *B. a. hemiophrys* site, several individual localities are designated in what is essentially a continous roadside ditch and adjacent pond complex. Within-year samples were generally small and have therefore been pooled by year. There are no obvious shifts in distribution between years or adjacent localities.

Locality 35 is a transition site. Roadside pools of the Brokenhead River where it is crossed by Highway 1, adjacent roadside ditches, and one adjacent dugout pond back from the roadside are included in this data. These almost certainly represent one breeding unit. There is some fluctuation between samples from either side of the mid-point particularly between the 11 May 1968 and the 22–23 May 1969 collections. The former only contains six males, however, and may not be representative. Comparison of the three 1968 samples (11 May, 14 May, 21 May) seems to rule out the possibility that *B. a. hemiophrys*-like individuals are breeding earlier and *B. a. americanus*-like ones later as the latest collection within the year (21 May) has the most intermediate sample. In the eight years spanned by the sampling (1962–1970), there seems to have been no shift toward either *B. a. hemiophrys* or *B. a. americanus*.

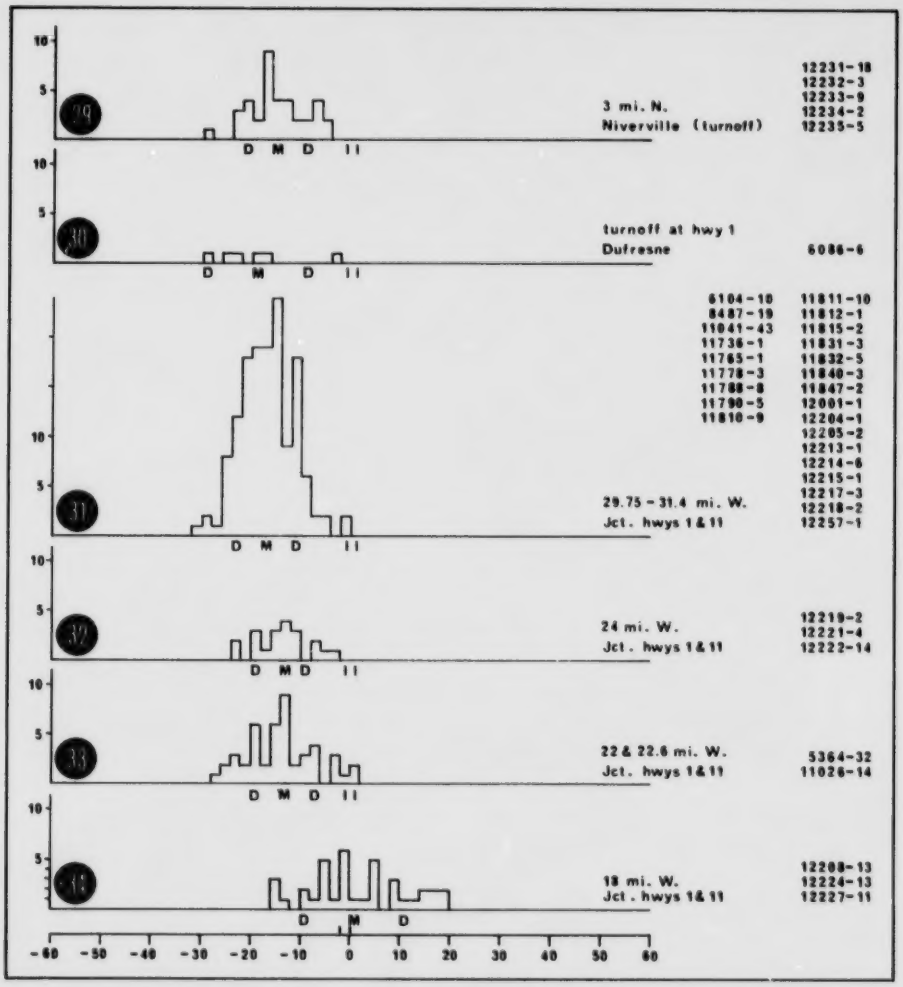


Figure 21. Histograms of individual discriminant scores of collections of male *Bufo* for collecting stations 29-34 along the western portion of the Trans-Canada Highway transect through southeastern Manitoba. An explanation of the letters, scale, etc. is given in the legend of Figure 10.

Locality 39 is an *B. a. americanus* site and represents roadside ditches along the Trans-Canada Highway spread along a distance of less than 1 mile [1.6 km]. The samples show little variation within a year or between years.

b. Univariate and Ratio Analysis of Males

The same characters examined individually or as ratios for geographic variation in the reference samples were computed for eastern Manitoba samples (Table 5).

Cranial Crest Score: Low mean values (1.0 and below) are evident to the west and high values (3.0) to the east. Values on or adjacent to the transition line plotted from the discriminant histograms are clearly intermediate and some intermediacy can be seen immediately east or west of this line.

Post-orbital Score: Values near 1.0 are evident in the west and near 2.0 in the east, but there is much more variability in this score than in the cranial crest value within *B. a. hemiophrys* than in *B. a. americanus* as earlier pointed out for the reference sample results, and it does not as clearly separate the taxa. However, the intermediate nature of populations within and to either side of the transition area is evident.

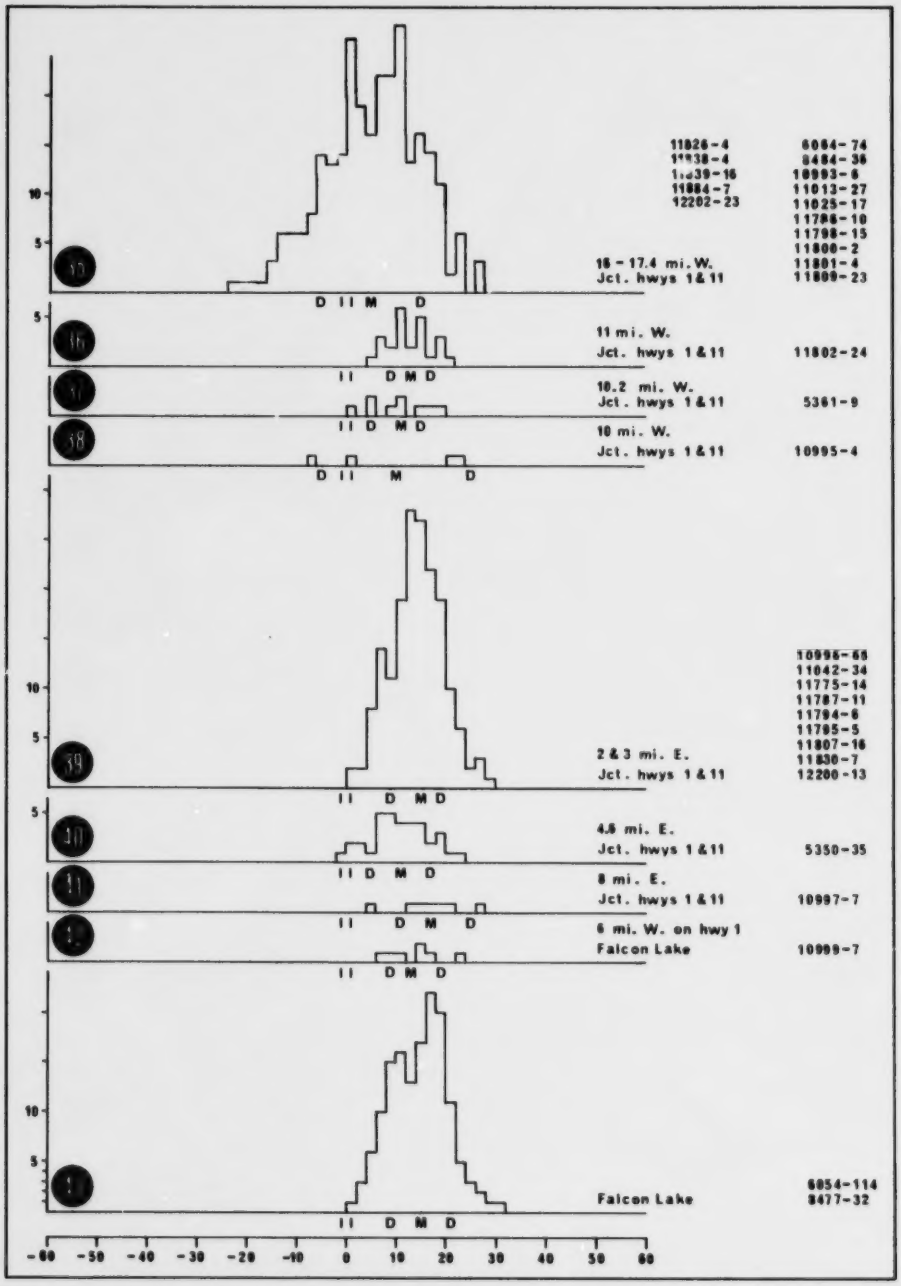


Figure 22. Histograms of individual discriminant function scores for collections of male *Bufo* for collecting stations 35–43 along the eastern portion of the Trans-Canada Highway transect through southeastern Manitoba. An explanation of letters, scale, etc. is given in the legend of Figure 10.

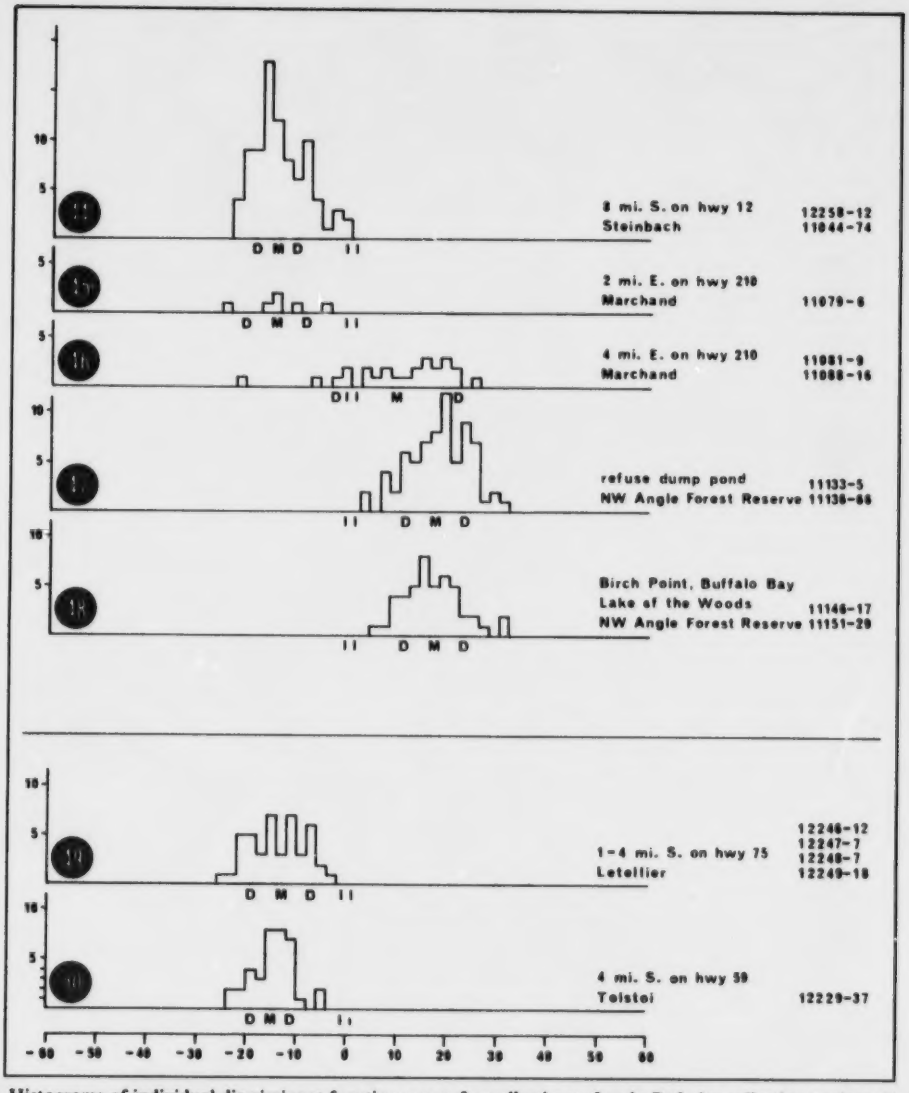


Figure 23. Histograms of individual discriminant function scores for collections of male *Bufo* for collecting stations 44–48 along the Marchand transect, and stations 49–50 along the western portion of the southern transect through southeastern Manitoba. An explanation of letters, scale, etc. is given in the legend of Figure 10.

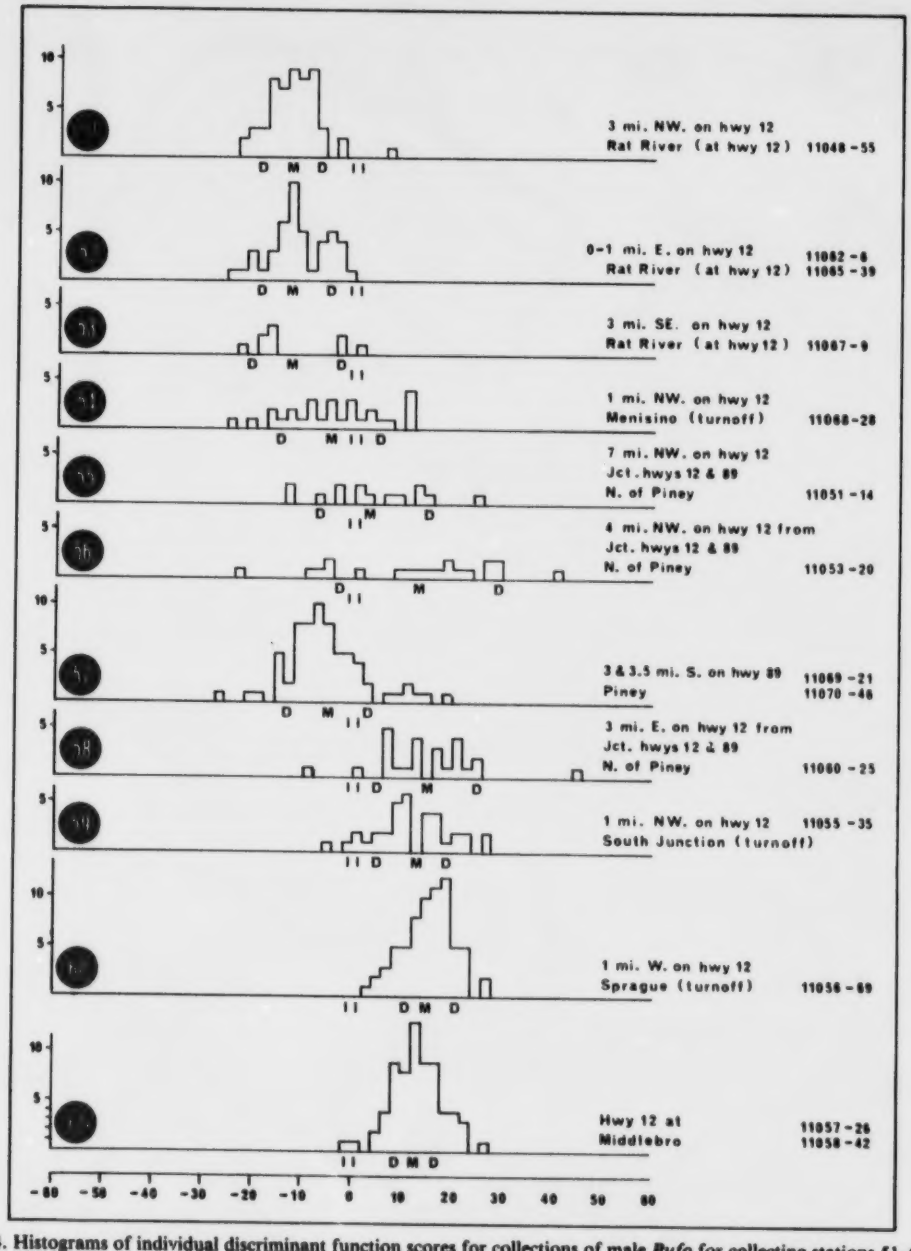

3 mi. NW. on hwy 12
Rat River (at hwy 12) 11048-55
0-1 mi. E. on hwy 12 11082-6
Rat River (at hwy 12) 11085-39
3 mi. SE. on hwy 12
Rat River (at hwy 12) 11087-9
1 mi. NW. on hwy 12
Manisino (turnoff) 11068-28
7 mi. NW. on hwy 12
Jct. hwys 12 & 89
N. of Piney 11051-14
4 mi. NW. on hwy 12 from
Jct. hwys 12 & 89
N. of Piney 11053-20
3 & 3.5 mi. S. on hwy 89 11069-21
Piney 11070-46
3 mi. E. on hwy 12 from
Jct. hwys 12 & 89
N. of Piney 11060-25
1 mi. NW. on hwy 12 11055-35
South Junction (turnoff)
1 mi. W. on hwy 12
Sprague (turnoff) 11056-69
Hwy 12 at 11057-26
Middlebro 11058-42
-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60

Figure 24. Histograms of individual discriminant function scores for collections of male *Bufo* for collecting stations 51–61 along the eastern portion of the southern transect through southeastern Manitoba. An explanation of letters, scale, etc. is given in the legend of Figure 10.

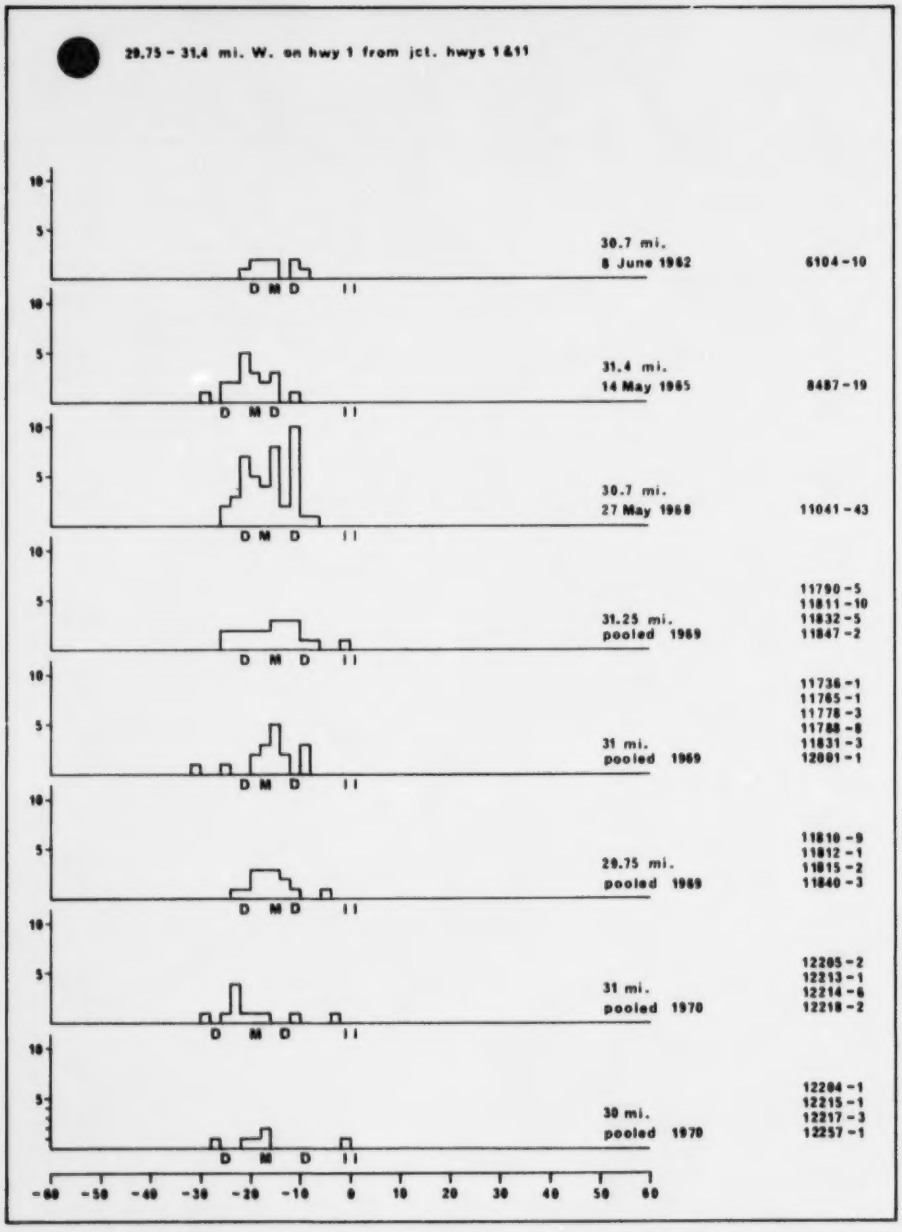


Figure 25. Histograms for individual discriminant function scores for collections of male *Bufo* from collecting station 31 in southeastern Manitoba to show variation in collections from adjacent sites and different years which were later pooled to form a combined sample for this station (see Figure 21). An explanation of letters, scale, etc. is given in the legend of Figure 10.

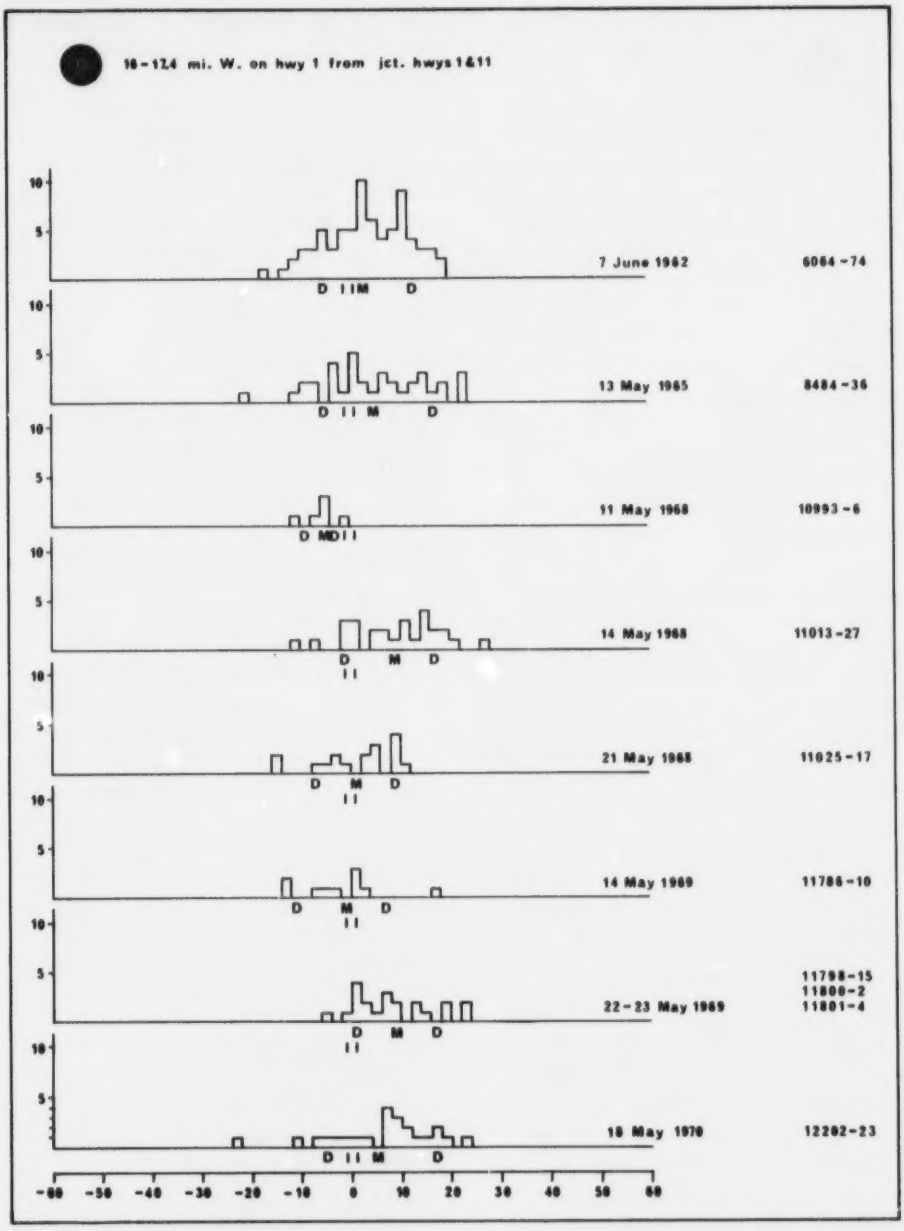


Figure 26. Histograms of individual discriminant function scores for collections of male *Bufo* from collecting station 35 in southeastern Manitoba to compare samples from different dates and years later pooled to form a combined sample for this station (see Figure 21). An explanation of letters, scale, etc. is given in the legend of Figure 10.

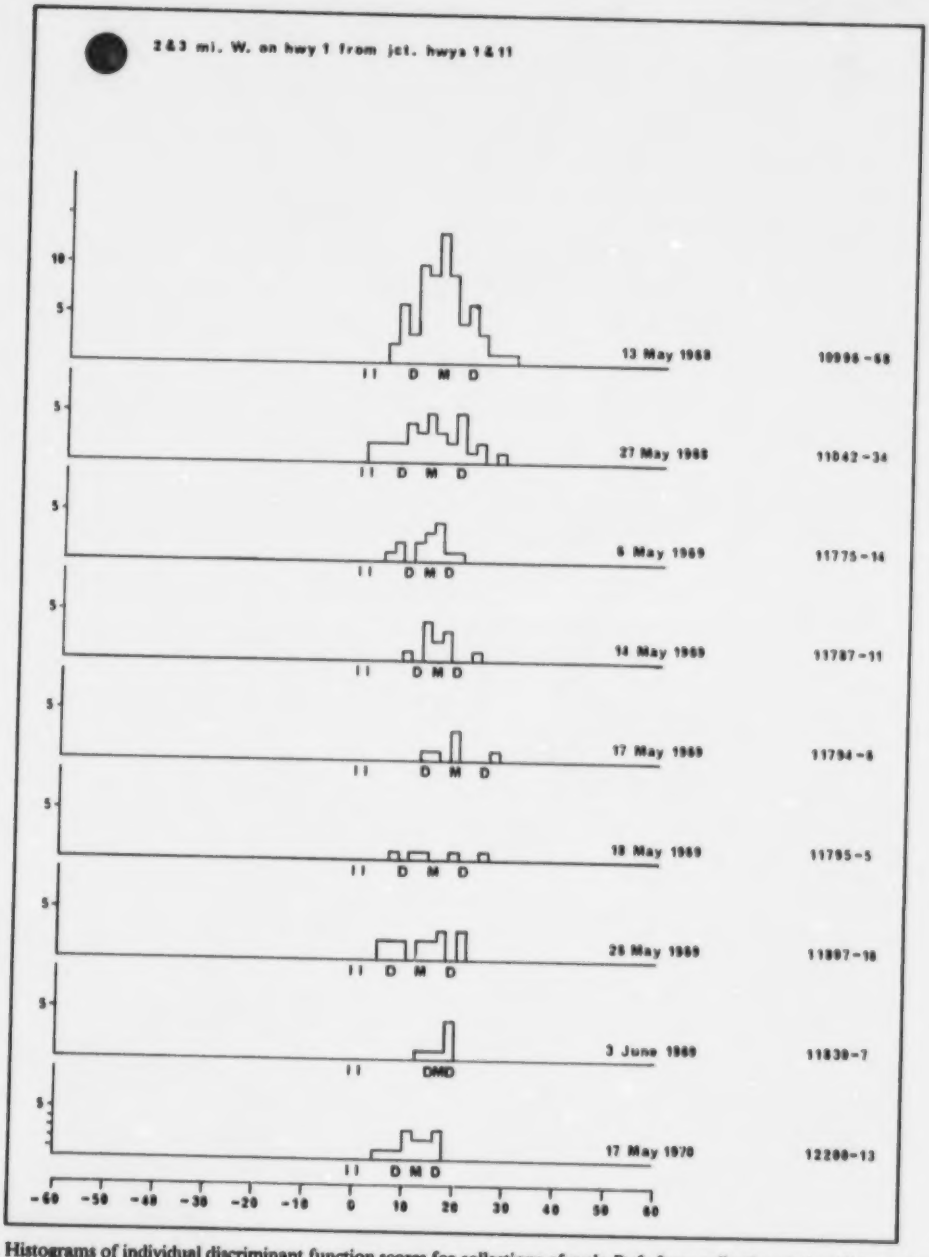


Figure 27. Histograms of individual discriminant function scores for collections of male *Bufo* from collecting station 39 in southeastern Manitoba from different dates and years later pooled to form a combined sample for this station (see Figure 21). An explanation of letters, scale, etc. is given in the legend of Figure 10.

Venter Score: In the reference sample comparisons, this character, as scored, did not separate *B. a. hemiophrys* and *B. a. americanus*. The variation between population samples across eastern Manitoba bears this out, but there is a marked increase in values in eastern Manitoba where *B. a. americanus* is present and more often has a completely spotted or reticulated venter (see Table 5). *B. a. hemiophrys* have generally lower values in this region than throughout their range and thus the transition zone does indicate a change in mean values from the low scores typical of western Manitoba *B. a. hemiophrys* to the higher scores of eastern Manitoba *B. a. americanus*. This holds true despite the fact that western populations of *B. a. americanus* (including those in eastern Manitoba) have somewhat lower venter scores than those in the eastern portion of the range.

Snout-vent Length: Values in the western portion of southeastern Manitoba are relatively low and this trend is most pronounced as the transition zone is approached. In the eastern portion the values are higher than in the west, but the body size increase is most pronounced along the transition line and falls off somewhat to the east. It is evident that there is a size decrease in *B. a. hemiophrys* populations, especially in the south where means for localities 44 and 50 are 45.6 and 43.5 respectively, the lowest mean size of all populations sampled throughout the study. The range of mature males varied between two collections pooled for the value of locality 44. A sample of 74 males collected 27 May 1968 ranged from 39.0 to 51.2, mean 44.7 mm, a sample of 12 taken at the same locality 12 June 1970 ranged from 48.8 to 56.0 mm. A sample of 37 males taken at locality 50 ranged from 38.1 to 47.9 mm. Generally in both species, population means to either side of the area of rapid transition were as large as any in eastern Manitoba, though greatly exceeded by some populations in the reference sample from the western portion of *B. a. hemiophrys* range and the eastern portion of *B. a. americanus* range.

Tibia/Snout-vent Length: Over the entire eastern Manitoba area the values range from 0.346 to 0.379, compared with the ranges of 0.351 to 0.385 in *B. a. americanus* and 0.341 to 0.381 in *B. a. hemiophrys* in the reference samples. There are some notably low scores in the western portion of eastern Manitoba (note locality 50 at 0.346), and these are often associated with low snout-vent measurements.

Spot Length/Snout-vent: Means in the western portion of the area are clearly higher, and fall into the range (0.144 to 0.175) of the reference sample for *B. a. hemiophrys*. Means in the eastern portion and lower portion of the *B. a. americanus* reference sample range are typical of southern and intermediate populations, though they are not as low as the Rondeau sample mean. The values on and near the transition zone are clearly intermediate.

Cranial Crest Posterior Width/Head Width: Values are clearly low in the western portion of eastern Manitoba and high in the eastern portion, with an abrupt shift over the transition line. In general the western values are in the high portion of the *B. a. hemiophrys* reference sample range whereas the eastern values tend toward the lower portion of the *B. a. americanus* range.

Outer Metatarsal Tubercle Width/Tarsus Length: Generally values are high in the western portion of the range and lower in the eastern portion of the range. Comparison with reference sample values of Table 3 shows the lack of a trend in the values within western Manitoba corresponds with the *B. a. hemiophrys* range in general but in the eastern portion the values approach the highest values of the character for *B. a. americanus*. The trend across the transition zone in eastern Manitoba is not as even as in some characters but the drop in ratio values is clearly evident, particularly to the west of the transition line.

c. Discriminant Function Scores for Females

The representation is meagre compared with male samples, and only 2–4 individuals from the majority of localities, but a few large collections were available. Figures 28 and 29 give the histograms of individual scores from each locality represented. Most localities show individuals to the left or right of the bulk of the sample giving an impression of more intermediate localities, but where a significant number of specimens are available, such as localities 31–35–39 along the Trans-Canada, the intermediate zone is clearly in the same position as it was for males. The results with females may be due to the smaller reference sample used, although females may actually be more variable. The tendency for *B. a. americanus* localities in eastern Manitoba to show some individuals over the mid-point of the axis toward *B. a. hemiophrys* is most evident in locality 27 and

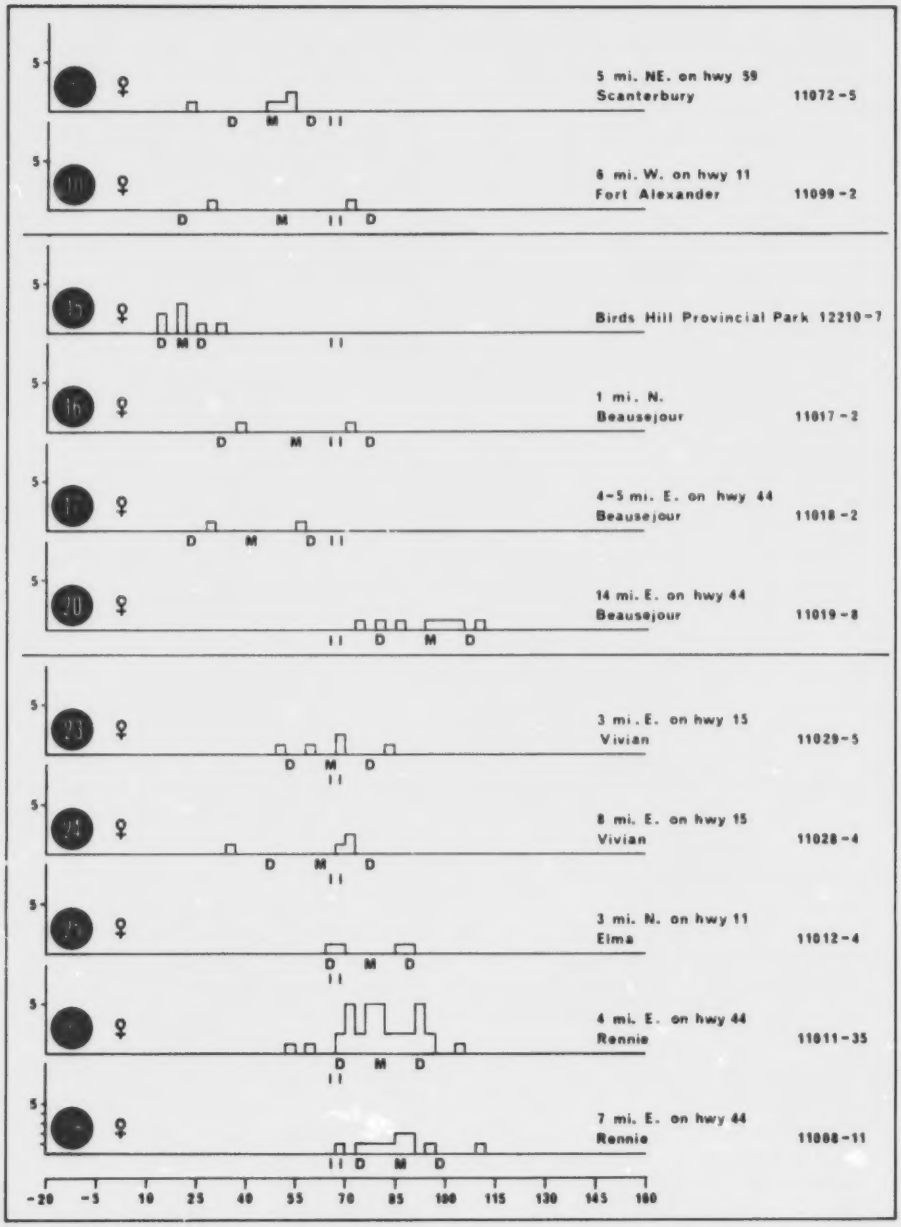


Figure 28. Histograms of individual discriminant function scores for collections of female *Bufo* for 11 collecting stations in southeastern Manitoba; 5–10 from northern transect, 15–20 from Beausejour transect, 23–28 from Vivian transect. An explanation of letters, scale, etc. is given in the legend for Figure 10.

locality 61, although neither of these has any individuals classifying as *B. a. hemiophrys* in the range of the reference sample (compare with Figure 10).

4. Breeding Call Analysis and Comparisons

Tape recordings were obtained in the field of the breeding calls of 223 individual male toads (Figure 30). In the laboratory three call parameters were determined: pulse rate, dominant frequency and duration. The call sampling was done mainly

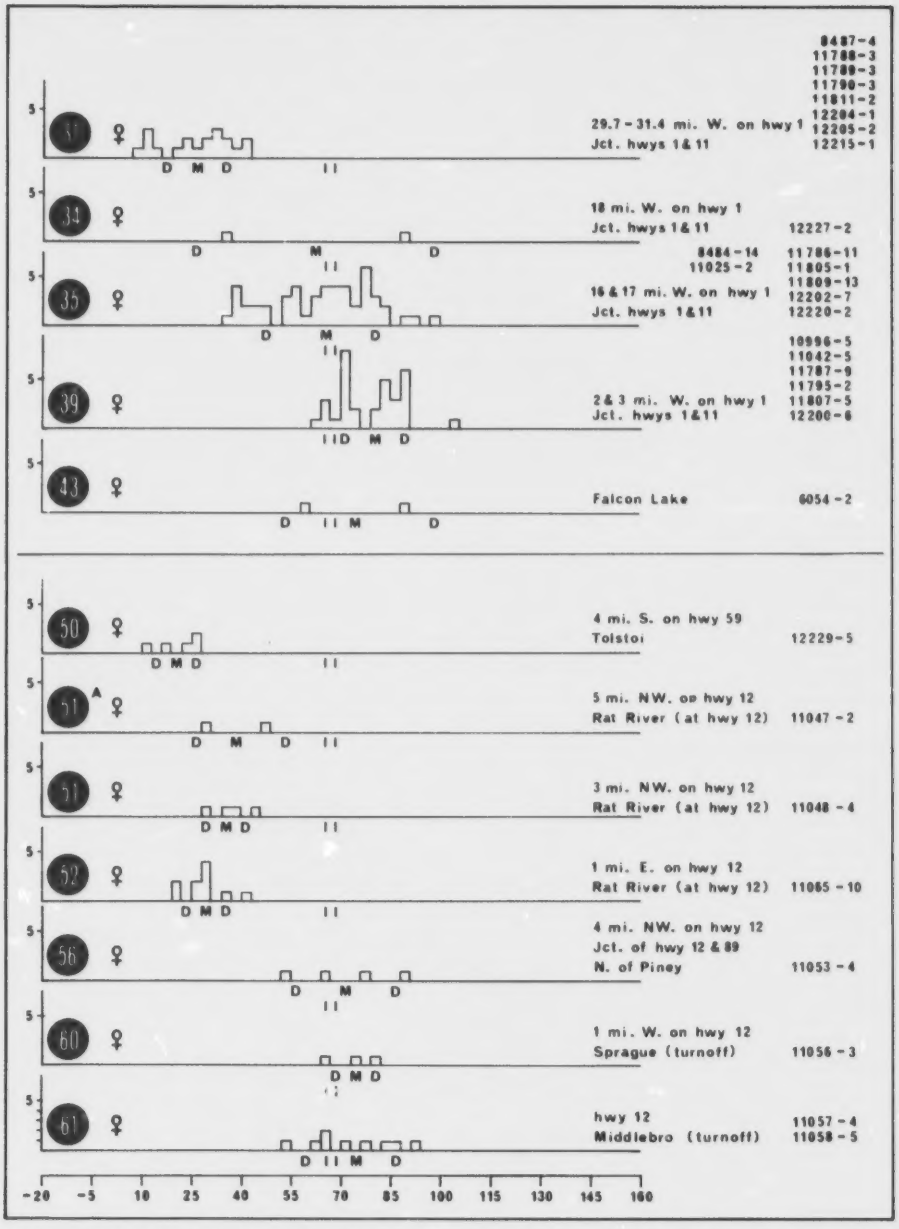
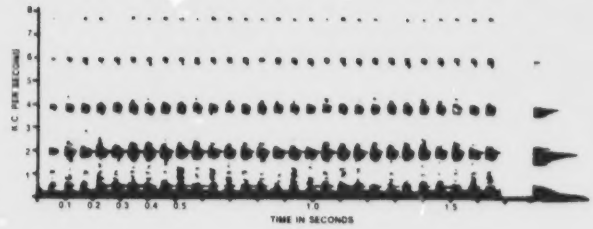


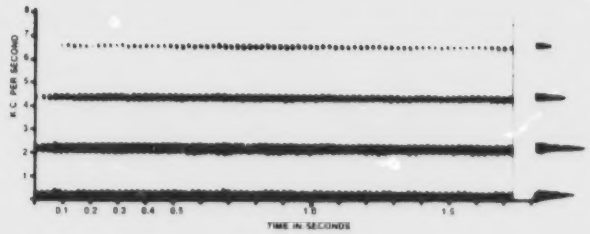
Figure 29. Histograms of individual discriminant function scores for collections of female *Bufo* for 12 collecting stations in southeastern Manitoba: 31–43 from the Trans-Canada Highway transect, 50–61 from the southern transect. An explanation of letters, scale, etc. is given in the legend for Figure 10.

along the Trans-Canada Highway transect, with additional populations sampled in the western half of eastern Manitoba as well as The Pas in western Manitoba and Oxdrift in western Ontario (Appendix II). The reference samples for both *B. a. hemiophrys* and *B. a. americanus* included populations from eastern Manitoba which are excluded from the morphological reference sample, and those in the "intermediate" sample included only individuals recorded 21 to 17 miles [33.8 to 27.4 km] west on Highway 1 of the junction of Highways 1 and 11.

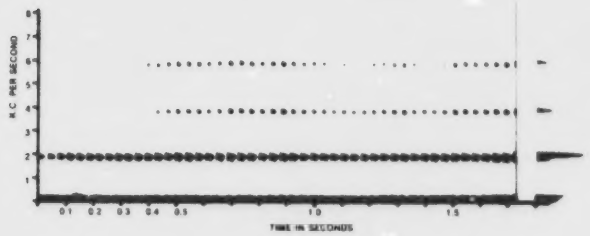
Figure 30. Audiospectrograms of portions of mating calls of male *Bufo americanus*. For each example a portion of a single call display is given on the left and a "section" showing relative energy at different frequency levels at one selected point in the call is given on the right. A, B and C are narrow band displays (45 cps filter) and D is a wide band (300 cps filter) display. The first display was used to determine pulse rate, the second to determine dominant frequency. Duration was timed from the tape.



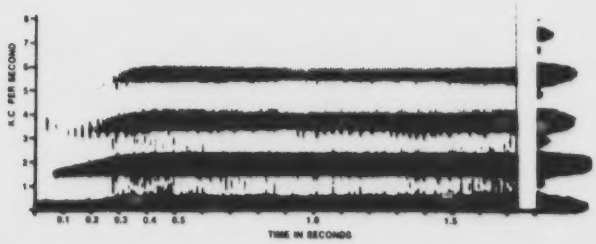
A. *B. a. americanus* NMC 11793-1 (tape 24): 3 miles [4.8 km] W on Highway 1 of junction of Highways 1 and 11, Manitoba, 16 May 1969. Body temperature of toad 10.0°C, pulse rate 17/sec, dominant frequency 1483.33, duration 11.0 sec.



B. *B. a. americanus* NMC 11807-1 (tape 34): 3 miles [4.8 km] W on Highway 1 of junction of Highways 1 and 11, Manitoba, 26 May 1969. Body temperature of toad 23.5°C, pulse rate 45/sec., dominant frequency 2083.33, duration 7.1 sec.



C. *B. a. hemiophrys* NMC 11831-1 (tape 39): 31 miles [49.9 km] W on Highway 1 of junction of Highway 1 and 11, Manitoba, 3 June 1969. Body temperature of toad 10°C, pulse rate 27/second, dominant frequency 1879.17, duration 5.7 sec.



D. *B. a. hemiophrys* NMC 12249-2 (tape 64): 1–2 miles [1.6–3.2 km] S on Highway 75 of Letellier, Manitoba. Body temperature of toad 23.5°C, pulse rate 103/sec, dominant frequency 1849.17, duration 2.1 sec.

Table 6. Correlation (*r*) and significance (*P*) values for comparison of variables in mating calls of *Bufo a. americanus*, *B. a. hemiophrys*, and "hybrid" populations.

	No. of Specimens	Pulse Rate		Call Length		Dominant Frequency	
A) Correlation with Temperature							
		r	*P*	*r*	*P*	*r*	*P*
B. americanus	50	+0.959	<<0.001	−0.635	<0.001	+0.416	<0.01
Intermediate pop.	62	+0.583	<0.001	−0.359	<0.05>0.01	+0.272	<0.05>0.01
B. hemiophrys	111	+0929	<<0.001	−0.459	<0.001	−0.036	>>0.1
B) Correlation with Snout-vent Length							
B. americanus	50	+0.223	>0.1	−0.170	>>0.1	−0.196	>>0.1
Intermediate pop.	62	−0.355	<0.01	−0.267	<0.05	−0.183	>0.1
B. hemiophrys	111	−0.304	<0.01	−0.018	>>0.1	−0.246	<0.05
C) Correlation with Pulse Rate							
				r	*P*	*r*	*P*
B. a. americanus	50			−0.653	<<0.001	+0.409	<0.01>0.001
Intermediate pop.	62			−0.588	<<0.001	−0.034	>>0.1
B. a. hemiophrys	111			−0.499	<<0.001	−0.027	>>0.1
D) Correlation between Call Length and Dominant Frequency							
				r		*P*	
B. a. americanus	50			−0.235		<0.0	
Intermediate pop.	62			+0.130		>>0.1	
B. a. hemiophrys	111			+0.031		>>0.1	

Table 7. Discriminant weights and the weight contribution to the Mahalanobis generalized distance from the discriminant analysis of one morphological (snout-vent length), one physiological (body temperature) and three call (pulse rate, dominant frequency and duration) parameters for *Bufo a. hemiophrys* and *B. a. americanus*.

Character	Discriminant Weight	Mean for *B. a. americanus*	Mean for *B. a. hemiophrys*	Weight Contribution
Snout-vent length	+0.95150	60.216	55.111	+4.857
Body temperature	+3.42561	14.989	19.440	−15.248
Pulse rate	−1.23113	27.644	74.622	+57.836
Dominant frequency	+0.00477	1871.340	1847.407	+0.114
Duration	+0.92707	11.172	3.448	+7.160
Mean discriminant score:		93.885	54.719	
Mahalanobis generalized distance: 39.166				

Table 6 gives the results of correlation tests between each possible pair of variables.

The number of pulses per second increased directly with an increase in temperature in both *B. a. americanus* and *B. a. hemiophrys* samples as shown by the correlation coefficients (0.959 and 0.929 respectively). The relationship of temperature and call length is inverse and the correlation is weaker (−0.635 and −0.459 respectively) than with pulse rate. There is considerable variation from call to call within a single individual at any given temperature and the value used here is an average of several calls from each individual. The relationship of dominant frequency to temperature

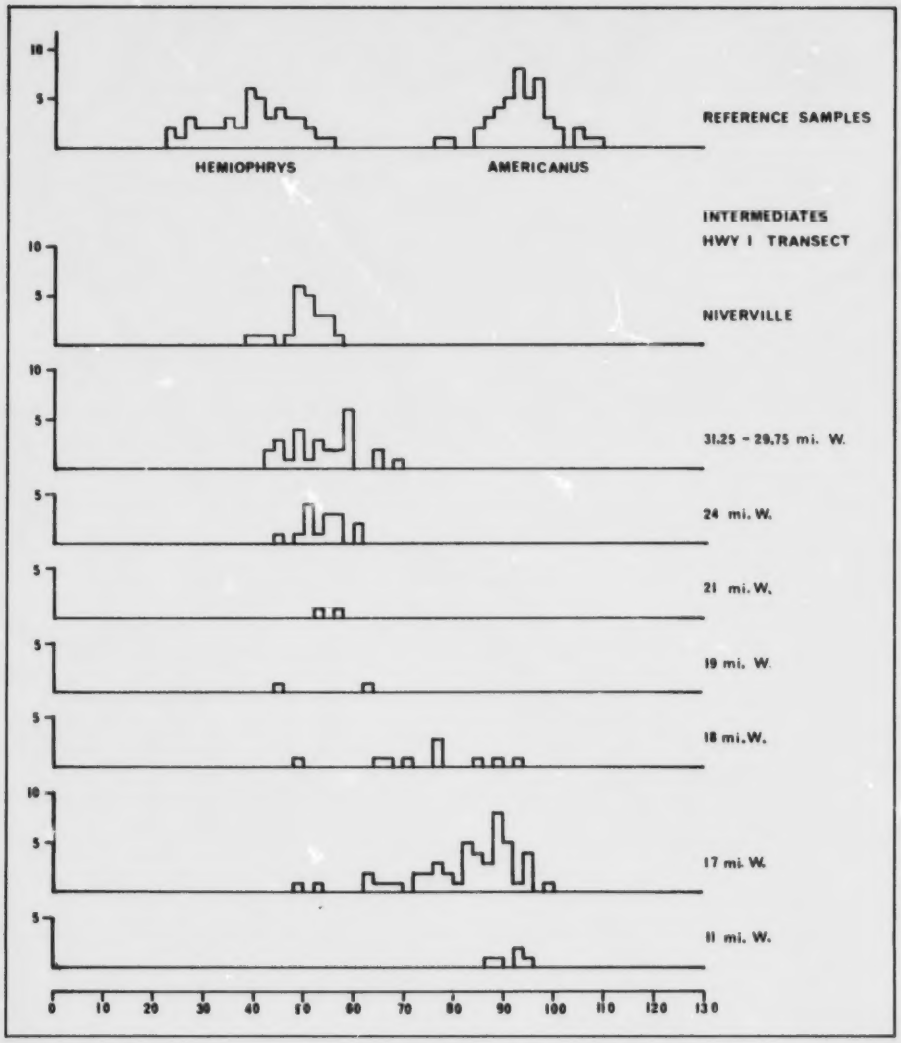


Figure 31. Histograms of call discriminant scores for individual *Bufo* in reference samples and in eight intermediate populations.

is positive and the correlation, although significant, is not high in *B. a. americanus* (+0.416, $P<0.01$) but negative and not significant in *B. a. hemiophrys* (−0.036, $P>>0.1$). Intermediate populations show weaker correlation between pulse rate (+0.583, $P<0.001$) and call length (−0.359, $P<0.05>0.01$) with temperature, and are intermediate in the relationship of dominant frequency and temperature (+0.272, $P<0.05>0.01$).

Weak positive correlation was present in *B. a. hemiophrys* between snout-vent and pulse rate (+0.304, $P<0.01$) but the correlation in *B. a. americanus* was not significant. In the intermediate populations the correlation was negative and weak (−0.335, $P<0.01$). Call length showed a weak correlation with snout-vent length in intermediates (+0.267, $P<0.05$) but was not significant in *B. a. americanus* or *B. a. hemiophrys*. Dominant frequency showed weak negative correlation and significance (−0.246, $P<0.05$) in *B. a. hemiophrys* but was not significant in *B. a. americanus* or intermediates. Note that dominant frequency was not correlated with temperature,

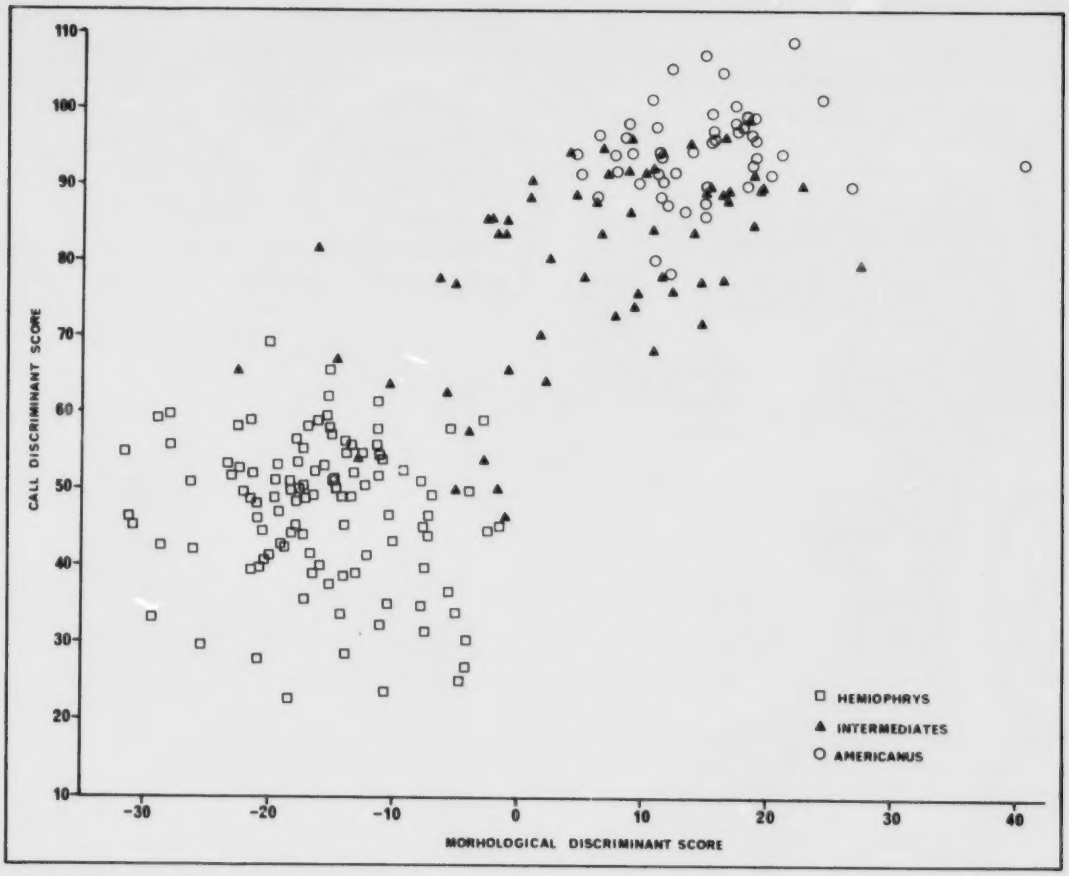


Figure 32. Relationship of call discriminant score to morphological discriminant score in three samples of *Bufo* (Appendix II gives localities and number of specimens).

but was weakly correlated with size, in *B. a. hemiophrys*.

Pulse rate correlation with call length was negative and weak but significant. Dominant frequency showed a weak positive correlation with pulse rate in *B. a. americanus* and an almost negligible negative correlation in intermediates, but was not significant in *B. a. hemiophrys*.

Call length in *B. a. americanus* showed a weak negative correlation with dominant frequency (-0.235, $P<0.01$) in *B. a. americanus* but no significant correlation in intermediates or *B. a. hemiophrys*. In *B. a. hemiophrys* in this study snout-vent length correlations with pulse rate (-0.304) and dominant frequency (-0.246) were significant ($P<0.01$, $P<0.05$ respectively).

Discriminant Analysis of Call Variables and Comparison with Morphological Discriminant Scores: A discriminant function using the three call variables (pulse rate, dominant frequency, and duration) as well as temperature and snout-vent length compared equal *B. a. hemiophrys* and *B. a. americanus* samples of 45 specimens each (see Appendix II). This analysis produced completed separation of the two taxa.

Application of the resultant weights (Table 7) to the individuals from geographically intermediate samples gave intermediate values (Figure 31). Pulse rate made the largest contribution to the separation between means (centroids).

Figure 32 plots the call discriminant vs. the morphological discriminant values for *B. a. americanus, B. a. hemiophrys* and intermediates. Table 8 gives results of correlation for each of the three units and the total sample. There is a positive cor-

Table 8. Correlation (r) and significance (P) values for comparison of morphological discriminant function and call discriminant function.

	No. sp.	r	P
Bufo americanus	50	+0.188	$>>0.1$
Intermediate populations	62	+0.552	$<<0.001$
Bufo hemiophrys	111	−0.178	$<0.1>0.05$
Pooled sample	223	+0.820	$<<0.001$

Table 9. Results of cross-mating and artificial rearing: number of individuals in each sample (50 eggs) which reached transformation.

Cross	Number reaching transformation		
	Laboratory	17 miles [27.4 km] west	31 miles [49.9 km] west
AA-1 (May 7)	45 (July 7-19)	11 (July 12-14)	11 (July 12)
AA-2 (May 16)	42 (July 8-Sept. 13)	4 (July 12)	22 (July 13-14)
AA-4 (May 27)	17 (July 14-24)	•	•
AA-5 (May 27)	44 (July 14-Aug. 25)	26 (July 14-16)	•
AI-4 (May 27)	40 (July 14-Dec. 13)	7 (July 15-16)	5 (July 14)
AH-1 (May 16)	47 (July 4-Aug. 11)	24 (July 13-16)	20 (July 13-18)
AH-4 (May 27)	18 (July 26-Oct. 2)	17 (July 15-19)	15 (July 13-17)
IA-5 (May 28)	23 (July 15-Aug. 9)	17 (July 14)	8 (July 14-Aug. 8)
II-1 (May 25)	39 (July 3-Oct. 5)	10 (July 14)	20 (July 13-17)
II-4 (May 27)	15 (July 11-Sept. 1)	7 (July 13-16)	•
II-5 (May 27)	3 (July 16-28)	4 (July 15-16)	10 (July 14-19)
II-6 (May 28)	37 (July 13-Aug. 31)	15 (July 14-16)	22 (July 14-29)
II-7 (May 29)	32 (July 18-Sept. 29)	10 (July 14-16)	28 (July 15-Aug. 7)
IH-1 (May 16)	11 (July 7-14)	16 (July 12)	23 (July 14-25)
IH-4 (May 27)	2 (July 11)	18 (July 13-29)	4 (July 14-16)
IH-5 (May 27)	18 (July 14-Aug. 25)	9 (July 14-16)	4 (July 13-14)
IH-6 (May 28)	21 (July 14-Sept. 3)	15 (July 14-17)	25 (July 13-15)
IH-7 (May 28)	26 (July 18-Nov. 12)	16 (July 14-16)	4 (July 14)
HA-1 (May 16)	3 (July 7-Aug. 13)	14 (July 13-21)	13 (July 14-26)
HA-2 (May 16)	12 (July 9-19)	12 (July 12)	26 (July 13-15)
HA-3 (May 16)	8 (June 30-July 12)	13 (July 13-14)	23 (July 13-16)
HI-2 (May 16)	11 (July 10-30)	7 (July 14-16)	24 (July 13-15)
HI-3 (May 16)	7 (July 4-12)	5 (July 12)	25 (July 13-21)
HH-4 (May 27)	6 (July 17-Aug. 25)	6 (July 14-15)	25 (July 14-31)
HH-5 (May 27)	12 (July 19-Sept. 16)	15 (July 14-21)	8 (July 15-29)

A *B. a. americanus* parent from 3 miles [4.8 km] west on Highway 1 of junction of Highways 1 and 11.
H *B. a. hemiophrys* parent from 31-29 miles [49.9-46.7 km] west on Highway 1 of junction of Highways 1 and 11.
I intermediate parent from 17 miles [27.4 km] west on Highway 1 of junction of Highways 1 and 11.

In each combination the first letter designates the female parent, the second the male; the number following the dash is the sequential number assigned a replicate within that cross combination. Missing numbers in sequence are crosses which failed to produce fertile eggs. Dates in parenthesis are: the date cross was made (after cross number) and dates of first and last individual to transform (after number reaching transformation).

• Indicates a prehatching failure at a particular locality due to accident or obvious contamination (e.g. fungal growth). In Figure 33 any replicate with a failure in laboratory or field is omitted in computing the mean.

relation (+0.820, P< <0.001) within the whole sample, and a correlation (+0.552, P< <0.001) for morphology and call within the intermediate populations. However, within *B. a. hemiophrys* or *B. a. americanus* there is no significant correlation between call and morphology, indicating they are varying independently within each taxon. These correlations (of total sample and intermediates) are evident despite the fact that a portion of the reference sample for call data includes populations where some introgression is evident in morphological characters, as demonstrated earlier.

5. Crosses Reared in Laboratory and Field

During the 1969 field season, males and females were selected from three sites along the Trans-Canada Highway: west of its junction with Highway 11: 31–29 miles [49.9–46.7 km] (*B. a. hemiophrys*), 17 miles [27.4 km] west (intermediate) and 3 miles [4.8 km] west (*B. a. americanus*). Crosses were attempted of every possible combination of males and females among these three populations (see Materials and Methods). In all, 61 pairings were tried but only 28 of these produced eggs, and in three of these the eggs did not develop, leaving only 25 successful crosses. However, they included at least one cross of every possible combination.

The number of individuals metamorphosing in each cross, the date the cross was made, and the dates of first and last transformation are given in Table 9. The mean number of individuals transforming from all complete replicates (with some transforming individuals in laboratory and both ponds) are compared in Figure 33.

In the laboratory, crosses using *B. a. americanus* females had high transforming success, and those using *B. a. hemiophrys* females had low success, with intermediate females yielding intermediate results. The water used in the laboratory was pumped in from Shoal Lake (personal communication, K.W. Stewart) in eastern Manitoba, an area well within *B. a. americanus* territory. No similar marked contrasts existed within or between the field-raised replicates. A Chi-square test of pure *B. a. americanus* and pure *B. a. hemiophrys* between intermediate (17 miles [27.4 km] w.) and *B. a. hemiophrys* (31 miles [49.0 km] w.) ponds gives a value of $\chi^2 = 1.30$, which is not significant ($0.5 > p > 0.1$). The experiment lacks sufficient successful replicates, is unbalanced

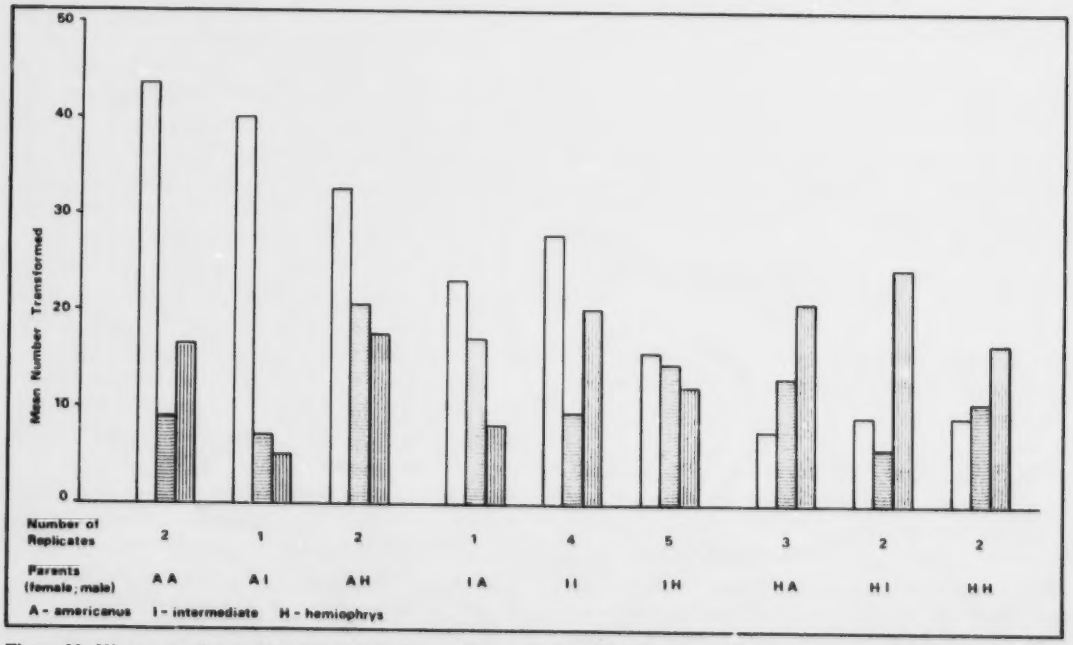


Figure 33. Histogram of mean number of tadpoles reaching transformation (from samples of 50 eggs) for each combination of *B. a. americanus*, intermediate, and *B. a. hemiophrys* parents in laboratory (open bars), in pond 17 miles [27.4 km] W on Highway 1 of junction of Highways 1 and 11 (horizontally hatched bars), and in pond 31 miles [49.9 km] W on Highway 1 of junction of Highways 1 and 11 (vertically hatched bars). The first letter given of each cross refers to the female parent, the second is the male.

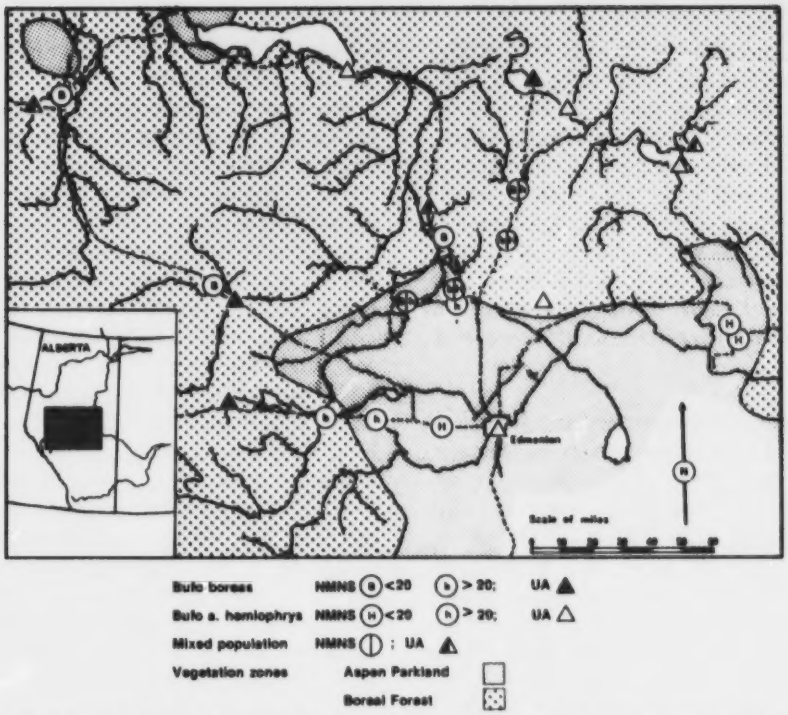


Figure 34. Map of west central Alberta showing the distributional relationship of *Bufo boreas* (B,b) and *B. a. hemiophrys* (H,h) in a narrow zone of sympatry. A capital letter indicates more than 20 males collected, a lower case letter, fewer than 20 males. All collections from breeding choruses. UA refers to collections in the University of Alberta, and NMNS to those in the National Museum of Natural Sciences.

because of unequal replicates between crosses, and shows wide variation in transforming success between some replicates (a difference of as much as 28 individuals transformed). In addition, some laboratory crosses took an abnormally long time to transform (e.g. to December 13 in AI-4!), possibly because of suboptimal temperatures and/or food.

6. Comparisons of *B. a. hemiophrys* and *B. a. americanus* with Other *Bufo* in Northern North America

a) ***B. a. hemiophrys–B. boreas:*** In 1965, the area north of Edmonton was examined during peak toad breeding and four breeding sites were found where *B. boreas* and *B. a. hemiophrys* were present in mixed choruses. Sites visited to the east of these contained only *B. a. hemiophrys* and those to the west only *B. boreas* (see Appendix I, Figure 34 and Table 10 for localities). Examination of the collection in the University of Alberta Zoology Department documented a slightly more extensive overlap zone (Figure 34).

In two of the mixed chorus sites examined, *B. boreas* was the more common species (but one of these contained only three calling males), in the other two *B. a. hemiophrys* predominated. Mismated pairs were found, usually a female of the less abundant species mated with a male of the predominant species. Only one individual suspected of being a hybrid was found, that in a pond (2.4 miles [3.9 km] north of Westlock) where *B. a. hemiophrys* was the more common species.

To compare the two species and evaluate the suspected hybrid, a discriminant comparison was made between 48 *B. a. hemiophrys* (NMC 8521) and 60 *B. boreas* (NMC 8530). The *B. a. hemiophrys* were from the same pond where the hybrid was taken, but because of the smaller sample of *B. boreas* available from this locality, a larger series from a nearby locality (2 miles [3.2 km] west on Highway 2 of Athabaska) was used for the reference sample of that taxon. Measurements were those taken for the *B. a. hemiophrys-americanus* comparison, except that all characters involving the cranial crest had to be

Table 10. Summary of localities where *Bufo boreas* and *B. a. hemiophrys* were found using the same breeding ponds in west-central Alberta during the 1965 field survey of the area.

	B. boreas		*B. a. hemiophrys*		*Hybrid*
	males	females	males	females	male
24 May 1965					
3 miles [4.8 km] W on Highway 18 of Freedom (turnoff)	2	0	1	0	0
26 May 1965					
2.4 miles [3.9 km] N on Highway 44 of Westlock	7	0	48	15	1
27 May 1965					
0.6 miles [1.0 km] SW on Highway 2 of Perryvale (turnoff)	8	1	36	0	0
28 May 1965					
2 miles [3.2 km] W on Highway 2 of Athabasca	60	5	4	1	0

Table 11. Discriminant weights and weight contributions to Mahalanobis generalized distance for each character for *B. a. hemiophrys* and *B. boreas*, mean discriminant scores for both species and values for a hybrid individual.

	Discriminant Weights	Mean for *B. a. hemiophrys* NMC 8521	Mean for *B. boreas* NMC 8530	NMC 8523	Weight Contribution
Snout-vent length	−4.842178	60.46243	62.88822	74.4	+11.74610697
Nostril separation	+29.637020	4.589577	3.9566610	4.5	+18.757774415
Nostril to parotoid (right)	+2.362891	11.87079	10.88994	13.8	+2.31641637
Nostril to parotoid (left)	−0.9124445	11.84996	10.93160	13.8	−0.837952531
Eyelid length	+1.16125	7.347894	7.429959	8.4	−0.095297981
Head width	+0.1174592	17.03535	17.29992	19.2	−0.031076181
Tympanum diameter	+11.6957	4.395826	3.734993	5.1	+7.728904518
Parotoid length (right)	+4.602726	10.9812	11.06492	13.1	−0.385340221
Parotoid length (left)	−4.331665	11.164530	11.10326	12.6	−0.265401115
Parotoid width (right)	+1.079502	6.929142	7.296618	8.2	−0.396691077
Parotoid width (left)	+2.935235	6.924962	7.326615	7.5	−1.178945943
Parotoid separation	+2.59703	9.358281	8.798274	11.1	+1.454354979
Spot length (right)	+1.009183	10.19787	9.464948	14.0	+0.739652423
Spot length (left)	−1.57778	10.19995	9.453274	12.5	−1.178090459
Spot width (right)	+0.3525454	4.904161	3.888327	4.2	+0.358127604
Spot width (left)	+1.497334	4.783328	4.029994	5.1	+1.127992612
Wart width (right)	−2.970933	2.204161	3.179994	3.0	+2.899134462
Wart width (left)	−2.052994	2.22916	3.373328	2.9	+2.348970039
No. warts per spot (right)	+0.05815297	5.1875	3.216666	4	+0.114609851
No. warts per spot (left)	+1.092478	4.791666	3.233332	6	+1.702445612
Tibia length	+5.659762	22.22492	21.93157	25.3	+1.660291183
Largest wart on tibia (right)	−1.178755	2.272912	11.67494	3.1	+11.08268752
Largest wart on tibia (left)	−1.918204	2.164577	11.913280	2.8	+18.70000109
Tarsus length	−4.341073	14.23952	15.18993	16.9	+4.125799190
"Spade" width	+21.52207	5.177079	3.496661	5.3	+36.16607383
Inner metatarsal tubercle width	−16.23179	2.208327	2.686661	2.3	+7.764217038
Mean discriminant score (centroid):		+106.9462	19.47962	+63.0288	
Mahalanobis generalized distance: 126.42582					

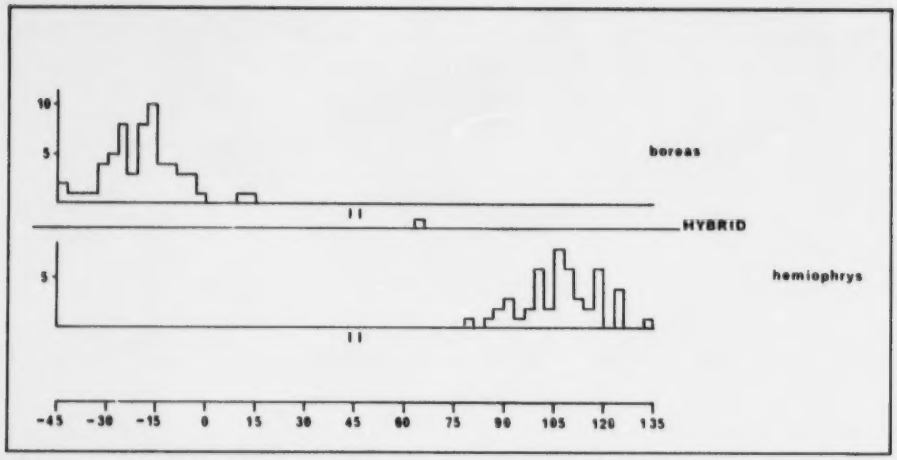


Figure 35. Histograms of discriminant function scores for reference samples of male *Bufo boreas* and *B. a. hemiophrys* and one natural hybrid between these taxa.

Table 12. Discriminant weights, weight contributions to Mahalanobis generalized distance, and means for each character for discriminant function analysis of *Bufo a. hemiophrys* and *B. cognatus*.

Character	Discriminant Weights	Mean *B. cognatus*	Mean *B. a. hemiophrys*	Weight Contribution
Snout-vent length	+1.9618	72.3333	63.0741	+18.165
Nostril separation	−10.0243	4.8292	4.6034	−2.263
Cranial crest length (right)	+10.1456	13.9979	11.9741	+20.532
Cranial crest length (left)	−0.9120	14.0417	12.0690	−1.799
Nostril to parotoid (right)	−3.0633	14.8313	12.9345	−5.810
Nostril to parotoid (left)	−1.2635	14.8958	12.9586	−2.448
Cranial crest width (anterior)	−21.5565	6.2792	5.5931	−14.789
Cranial crest width (posterior)	+17.8171	9.1292	4.6121	+80.481
Eyelid length	−2.2461	8.5875	7.8138	−1.738
Head width	+2.5182	21.1250	17.8052	+8.360
Tympanum diameter	+1.1682	5.0458	4.7552	+0.340
Parotoid length (right)	−4.1194	10.7563	11.3224	+2.232
Parotoid length (left)	+3.2425	10.6896	11.4759	+2.550
Parotoid width (right)	−5.9609	6.7646	7.1259	+2.154
Parotoid width (left)	+2.5110	6.6667	6.9828	−0.794
Parotoid separation	+0.2284	13.9333	10.1948	+0.854
Spot length (right)	−0.2942	16.4396	9.8328	−1.944
Spot length (left)	−0.0370	14.9000	9.3328	−0.206
Spot width (right)	+4.0041	7.6417	4.4724	+12.690
Spot width (left)	−0.9240	7.3875	4.4052	−2.756
Wart width (right)	+10.2861	2.0854	2.1328	−0.487
Wart width (left)	−2.9931	2.1104	2.1138	+0.010
No. of warts per spot (right)	+0.4006	28.2083	3.500	+9.898
No. of warts per spot (left)	+0.4401	25.7500	3.6207	+9.740
Tibia length	−2.7946	25.6970	23.3534	−6.552
Largest wart on tibia (right)	+0.5363	2.5042	2.2966	+0.111
Largest wart on tibia (left)	+1.5136	2.6312	2.4224	+0.316
Tarsus length	+2.4015	15.9792	14.4948	+3.565
"Spade" width	+3.7639	6.3917	5.4586	+3.512
Inner metatarsal tubercle width	+24.3866	2.9250	2.1724	+18.353
Mean discriminant scores (centroids):		249.605	97.228	
Mahalanobis generalized distance: 152.377				

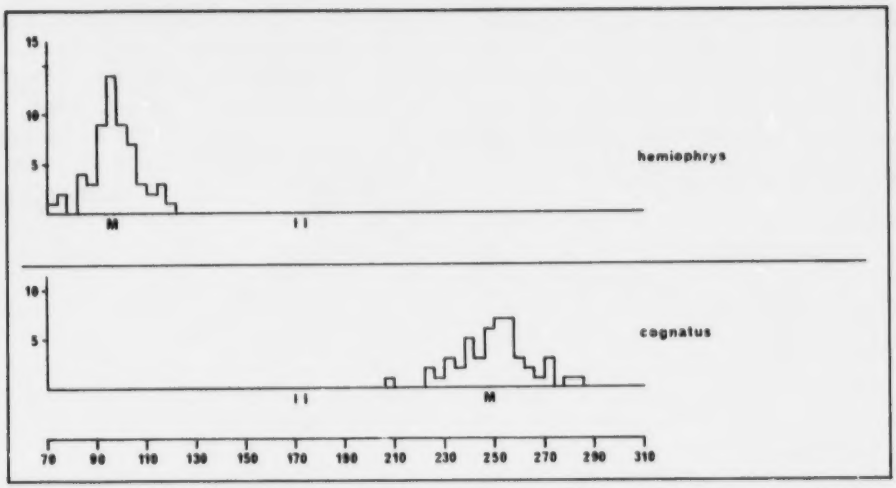


Figure 36. Histograms of discriminant scores for reference samples of male *Bufo a. hemiophrys* and *B. cognatus*.

omitted since *B. boreas* usually lacks crests, or has crests so low that they are barely discernible and rarely can be measured with confidence. Several other characters which distinguish *B. boreas* and *B. a. hemiophrys* were not included in this comparison. Besides the lack of cranial crests, *B. boreas* also lacks a dark throat in the male, and possesses a prominent tarsal fold.

The discriminant weights, weight contributions to the Mahalanobis generalized distance, means for *B. a. hemiophrys* and *B. boreas* samples, and the measurements for the suspected hybrid are given in Table 11.

The weight contributions indicate that "spade" width, nostril separation, size of tibia wart, body size, inner metatarsal tubercle width, tympanum diameter and tarsus length are making the largest contribution to separating the taxa in the analysis. *B. boreas* has a smaller spade, a larger inner metatarsal tubercle, a much larger tibial wart, somewhat larger snout-vent, smaller tympanum diameter and a longer tarsus. The mean of the discriminant scores for *B. a. hemiophrys* was +106.9462 (+78.16148 to +132.4602) and for *B. boreas* +19.47962 (+44.11512 to −14.2396). The suspected hybrid had a score of +63.0288. Histograms of the discriminant scores are given in Figure 35.

In addition to its intermediate score the hybrid had distinct, but open, cranial crests, with no indication of a *B. a. hemiophrys*-like central filling or posterior bridging. The anterior width of the cranial crest was 4.9 mm and the posterior 6.1 mm and on the *B. a. americanus-hemiophrys* boss scoring system it would be assigned a score of 3, clearly distinguishing it from the 0 or 1 score normal for a *B. a. hemiophrys*. The crest measurements, posterior wider than anterior, are the reverse of those typical for *B. a. hemiophrys*. Postorbital crests were absent and therefore scored 0. The venter scored 5. The hybrid lacked the tarsal fold typical of *B. boreas*. Although the spot length does not differentiate the two species in this analysis, *B. boreas* often has spots that run together, in contrast to the distinct blotches typical of many *B. a. hemiophrys*.

b) ***B. a. hemiophrys–B. cognatus:*** Only one mixed breeding chorus of *B. a. hemiophrys* and *B. cognatus* was found in the course of herpetofaunal surveys of the Canadian Prairie Provinces, but this was the only time when any breeding aggregation of *B. cognatus* was found during these surveys. *B. cognatus* breeds typically after heavy rains. On 28 May 1963 a large prairie slough 8.3 miles [13.4 km] east on Highway 1 of the Piapot turnoff yielded 48 male and 4 female *B. cognatus* and 22 male and 2 female *B. a. hemiophrys*. No mismated pairs were noted.

B. cognatus is morphologically distinctive from *B. a. hemiophrys*. Males in the Piapot series ranged from 66.1 to 76.4 mm snout-vent length. Particularly obvious were the characteristically heavy cranial crests which unite on the nose and diverge strongly posteriorly. The postorbital crests are strongly developed, and the large, prominent dorsal blotches each contain 14 to 54 small warts. The outer metatarsal tubercle is large and broad.

The venter is slightly spotted to unspotted (score values of 3(2), 2(12), and 0(34) among the males).

A discriminant analysis was made between the 48 male *B. cognatus* from Piapot (NMC 7110) and 22 male *B. a. hemiophrys* from Piapot (NMC 7111) pooled with an additional 36 males from nearby Tompkins (NMC 6014). The discriminant weights, weight contributions, and mean values for each character are given in Table 12 and the discriminant scores presented as a histogram in Figure 36. This analysis confirms the distinctiveness of the two taxa and the lack of intermediates where they are sympatric.

The largest contributions to the separation between these taxa are made by the posterior cranial crest, cranial crest length, inner metatarsal tubercle, snout-vent length, spot width and number of warts per spot. *B. cognatus* is a larger toad than *B. a. hemiophrys*, with much greater posterior divergence of the cranial crest, larger spots with more warts per spot, and a somewhat larger spade.

c) *B. a. hemiophrys–B. w. woodhousei* and *B. a. americanus–B. w. fowleri*: Two small series, one each of *B. w. fowleri* (19 males taken 19 June 1973 at Long Point, Norfolk County, Ontario) and *B. w. woodhousei* (10 males 22 June 1965 and 7 males 23 June 1965 from 3.5 miles [5.6 km] southeast of Stockton, Rooks County, Kansas) were available for comparison (see Appendix I).

The weights produced by the discriminant separation of *B. a. americanus* and *B. a. hemiophrys* were applied to *B. w. woodhousei* and *B. w. fowleri* specimens to determine where they would rank in comparison with these taxa. The results are given as histograms in Figure 37. Interestingly, they both score as intermediate between *B. a. hemiophrys* and *B. a. americanus*. Both subspecies of *B. woodhousei* produced nearly identical scores on the basis of the characters used. The variance in these samples, however, is low in contrast to the generally high variances of populations in the *B. a. americanus* and *B. a. hemiophrys* contact zone. Other characters, which are not included in this comparison, more-or-less separate the subspecies of *B. woodhousei* and more effectively separate each from *B. a. americanus* and *B. a. hemiophrys*.

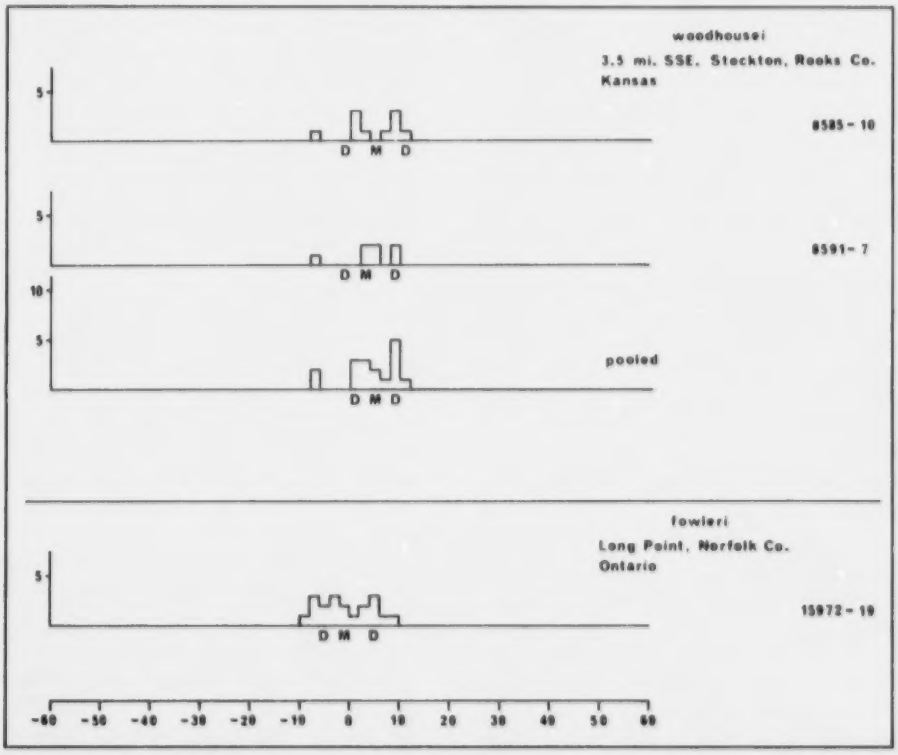

woodhousei
3.5 mi. SSE. Stockton, Rooks Co. Kansas
8585 - 10
8591 - 7
pooled
fowleri
Long Point, Norfolk Co. Ontario
15972 - 19
D M D
-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60

Figure 37. Histograms of discriminant scores for two samples of male *Bufo woodhousei*. Weights produced by the *Bufo a. americanus–hemiophrys* discrimination were used to complete these values.

Discussion

1. Habitat

a) Breeding Habitat

In general, populations of *B. a. americanus* seemed to be larger and males more clustered. In contrast, it was common to find *B. a. hemiophrys* males more dispersed, often in scattered small clumps of only two or three calling together. This contrast becomes obvious on examining the sample sizes in collections of the two taxa. Partly, this difference in the field is due to the fact that ponds are often larger and deeper in boreal forest and relatively smaller and shallower in aspen parkland and grassland. On the subjective basis of field experience throughout the range of both species, the impression exists that toads in the eastern part of northern North America gather in more extensive and larger breeding aggregations than they do in the central region.

b) Summer Habitat

Breckenridge and Tester (1961) reported from intensive studies of *B. a. hemiophrys* in Minnesota that it was found most often in or near the margins of prairie ponds. Field observations of *B. a. hemiophrys* over its Canadian range do not strongly support this as typical of *B. a. hemiophrys* populations in general. Samples of *B. a. hemiophrys* (such as the one through the Spruce Woods Forest Reserve in July 1960) have been taken of many individuals foraging well away from water. Surveys made in the summers of 1959–1967 along the margins of lakes, ponds, sloughs, and potholes of all types throughout the range of *B. a. hemiophrys* seldom revealed large numbers of post-breeding or post-metamorphosing *B. a. hemiophrys* at such sites. Exceptions are the Wascana marsh at Regina, Saskatchewan, and the Delta marsh in Manitoba, where large summer series of all sizes of foraging *B. a. hemiophrys* were taken. Single adult *B. a. hemiophrys* were occasionally present at pond margins but usually occur there at the end of the breeding season. However, a large series of *B. a. americanus* of all sizes was taken along a lake margin in eastern Manitoba in August, and occasional adult *B. a. americanus* were found at pond margins near the end of breeding activity. If there is a tendency for *B. a. hemiophrys* to be restricted to pond margins more often than *B. a. americanus* it is not clearly indicated by present field data.

2. Morphology

a) Non-quantitative

Live *Bufo a. americanus* and *B. a. hemiophrys* are readily distinguished in the field, but their differences are hard to quantify. Colour characteristics, not treated quantitatively, are helpful in this respect. *B. a. americanus* is usually more brightly coloured, with backgrounds of red, brown, yellow or green. *B. a. hemiophrys* is a more drab toad, usually greyish or dull brownish. A rusty-coloured phase occurs (Cook 1964c) but it is more subdued than the red of *B. a. americanus*. Other good field characters, such as distribution of warts, spot size and cranial crest features are treated in detail in the morphological analysis. The difference between the calls of breeding males makes the two taxa easily distinguishable to the ear.

In field sampling across the east-west transects of the interbreeding zone the variety in morphology and vocalization gives the impression of sleight-of-hand by a master conjurer. In eastern Manitoba one can collect what appears to the eye and to the ear as "good" *B. a. americanus*, as recognizable as any from Prince Edward Island to western Ontario. If one misses the intermediate zone, then in the Aspen Parkland east of the Red River one can sample "good" *B. a. hemiophrys*, as recognizable as any from central Manitoba to western Alberta. When the gap between the two is sampled, a point of change between breeding sites is subtle and almost imperceptible.

b) Quantitative

A major problem in any morphological comparison is finding an objective and practical method to evaluate and contrast variations. The discriminant function analysis used here has produced a high-resolution, repeatable analysis of morphological variation over the range of the parent taxa and through the transition zone between them.

The reference samples represent a good geographic dispersal for *B. a. hemiophrys*. Although they are relatively weak in material from

North Dakota and the northern portions of Alberta and Saskatchewan, all major habitat types which its range covers are represented. The Laramie Valley (Wyoming) disjunct population is also included. The sample largely avoids the southern periphery of the continuous range of *B. a. hemiophrys* and possible influence from the presumably allopatric *B. w. woodhousei*. The sample of *B. a. americanus* is drawn entirely from the northern portions of its range, largely to avoid its extensive area of geographic sympatry over the eastern United States with *B. w. fowleri*. Only two localities, Rondeau and Point Pelee parks in southern Ontario, are within that contact area. This restriction on the geographic area of samples used means that the discriminant analysis does not represent total *B. a. americanus* variation. However, the samples do cover three major habitat areas occupied by *B. a. americanus* in the northern part of its range. Western Ontario is the least well represented geographic area. Encompassed by the samples are areas supposedly occupied by the northern race *B. a. copei* (Logier and Toner 1961; Ashton, Guttman and Buckley 1973; Conant 1975) and the transition between it and *B. a. americanus*.

In general, the variation in population mean discriminant values within taxa does not markedly follow geographic clines or forest zones though some trends are pointed out in the Results section. However, the wide area of low western scores for *B. a. americanus* and narrow area of high eastern scores for *B. a. hemiophrys* may be of some importance (see below). Most previous comparisons of *B. a. americanus* and *B. a. hemiophrys* (Breckenridge 1944; Blair 1957a; Underhill 1961) have agreed that these taxa are as morphologically distinct as good species, but sample size, geographic representation and number of characters have not been very extensive.

A post-orbital score was used by Henrich (1968) as part of his basis for separating the two taxa. It is clearly shown here from the much more extensive geographic coverage of *B. a. hemiophrys* that post-orbital crests may be present on individuals well within *B. a. hemiophrys* range and neither their presence nor that of the intermediate conditions clearly establish an individual as intermediate.

The ventral scoring used here unfortunately encompasses only part of the ventral pattern variation exhibited in the two taxa, since it deals solely with the area covered by pigment. As Henrich (1968) pointed out, there is a tendency for *B. a. hemiophrys* to have lighter ventral markings, and he used a scoring system which attempted to evaluate this difference. However, there is a large subjective element in any attempt to score relative intensity of markings, and this is especially true of trying to assign scores to intermediate specimens. Furthermore, preservation may affect collections differently and there is a danger of scoring preservation differences rather than real phenotypic ones. Similarly, the sometimes obvious difference between individuals in the relative size of markings, spots or reticulations, has not been included in this assessment because of difficulties in obtaining objectivity. Differences are most obvious in the northern populations of *B. a. americanus* and this character does not seem to have a prime importance in the general *B. a. hemiophrys-americanus* comparison.

It is notable in the ventral score results that northern *B. a. americanus* populations score high, southern score low. The tendency of Prince Edward Island toads to have a high ventral score has been noted previously by Cook (1967). The suggestion of Bleakney (1952) that there is a difference between Annapolis Valley populations and those outside this valley within Nova Scotia is not evident in comparing Wolfville (valley) and Musquodoboit (Halifax County) samples here.

There is great variation in mean snout-vent length in both taxa. The largest population mean lengths (71.8 for *B. a. americanus* and 70.8 for *B. a. hemiophrys*) are surprisingly close, although the smallest means (53.9 and 45.8 respectively) are more disparate. When individual maxima and minima are examined, 88.4 vs. 79.1 and 42.1 vs. 41.3 for *B. a. americanus* vs. *B. a. hemiophrys* respectively, the agreement is closest in the smallest size range. The discriminant analysis has apparently responded to this variation by giving little weight to size in separating the taxa. The potential minimum size at maturity, and the maximum size attainable are, evidently, not that different between the two taxa. Much remains to be understood about geographic variation in size in toads, and the influence of local ecologic conditions and yearly variations in rainfall and temperature and therefore on the length of the effective growing period. Perhaps abundance of food and the size at transformation and the total hours per year of optimum foraging weather (in terms of temperature and moisture) have an important bearing on the question. Clear north-south or east-west gradations in either taxa are not ap-

parent in the present samples, although there is a generalized trend for *B. a. hemiophrys* to be smaller in the east, and *B. a. americanus* to be large in the south and smaller in the north.

The relative tibia length has been thought to decrease toward the north in some anurans (Schmidt 1938; Bleakney 1974). This is not clear-cut in *B. a. hemiophrys* or *B. a. americanus* populations sampled here, although there may be a trend in this direction in *B. a. americanus*. Jameson *et al.* (1973) have suggested for the Pacific Tree Frog (*Hyla regilla*) that various proportions are correlated with local climatic conditions.

The relative length of the spot (spot length divided by snout-vent length) is much more variable in *B. a. americanus* than in *B. a. hemiophrys*. Western and southern *B. a. americanus* populations have a smaller mean spot size than *B. a. hemiophrys*, though the entire *B. a. americanus* range encompasses the variation in *B. a. hemiophrys*. It is also noteworthy that there is a difference in dorsal spotting between northern *B. a. americanus*, where large (and often irregular) spots are present, and southern (and western) populations where smaller spots are more typical. The fact that western *B. a. americanus* are small spotted argues against the large spots in northern *B. a. americanus* being derived from contact with *B. a. hemiophrys*.

The separate discrimination for reference samples of females, though based on much smaller numbers (59 *B. a. americanus* and 118 *B. a. hemiophrys*) than for males, also separated the two groups completely. The differences in weighting may be due to both the smaller sample and the very uneven geographic distribution of female samples (see Appendix I). The Mahalanobis generalized distance between *B. a. americanus* and *B. a. hemiophrys* is 104.66 in the female analysis compared with a mean of 51.35 in the male discrimination, possibly indicating that larger body size accentuates the differences in measurements.

The discriminant scores for males from southeastern Manitoba clearly define an obvious zone of populations which are intermediate in mean value and have a wider dispersal of scores (as shown by the high variance values) between reference sample populations of *B. a. americanus* and *B. a. hemiophrys*. This zone is only a few kilometres wide, and its narrowness most sharply demonstrated on the Trans-Canada transect where intensive sampling indicates it is most pronounced over 10 miles [16 km] or less in width. The narrowness of the zone is also apparent in the other five transects. The histograms show no clear indication of modality in the distribution of scores which would have been indicated in hybrids mixed with "pure" examples of either or both parental types. Instead, the distribution seems best interpreted as complete interbreeding and backcrossing with no segregation into *B. a. americanus* or *B. a. hemiophrys* types within the interbreeding zones.

The lowered scores east and west of the most obvious interbreeding zone could be interpreted as due to convergence in each taxon toward characters of the other as the intermediate zone is approached. This interpretation would require selection to be acting on the natural variation within each of the *B. a. hemiophrys* and *B. a. americanus* stocks and would ignore the likelihood of gene flow from the intermediate zone. Discriminant scores of *B. a. hemiophrys* do not tend toward those for *B. a. americanus* over the northern part of the former's range where it occurs in boreal coniferous forest, nor do scores of northeastern *B. a. americanus* tend toward *B. a. hemiophrys* where *B. a. americanus* also occurs in boreal coniferous forest. Because the lower scores grade relatively smoothly toward the area of contact, it is evident they reflect the direct effects of introgression from the contact area. This distribution of lowered mean scores only as far as Delta in central Manitoba, but all the way to Whitetop Creek, northeast of Moosonee in central Ontario, suggests that introgression is more successful into *B. a. americanus* than into *B. a. hemiophrys* populations. That this is really occurring is further indicated by the observation of Logier (1928) of a specimen with "temporal crests" (cranial crests) resembling *B. a. hemiophrys* in a sample of toads from Lake Nipigon (geographically between Oxdrift and Whitetop Creek samples analysed here).

Characters analysed separately or in ratios also show the same pattern of abrupt change over a narrow zone that is evident in the multivariate analysis. Cranial crest score, cranial crest posterior width/head width, and "spade" width/tarsus length show the abrupt change in their means across this zone, as does the spot length/snout-vent length ratio. The latter value is different between western *B. a. americanus* and all *B. a. hemiophrys*. It did not weight heavily in the

discrimination, but this may have been because of its broad variation over the whole of *B. a. americanus* distribution.

The scores for females from southeastern Manitoba show a similar pattern to that obtained with the scores for males though the narrowness of the transition zone between the two taxa is not as well defined by the former. This may be partly due to the meagre samples of females available, as the largest samples show the greatest agreement with the results obtained for males.

c) Selective Advantage of Taxonomic Characters

B. a. hemiophrys has a major portion of its range in Aspen Parkland and Prairie regions and is therefore subject to more arid conditions and greater environmental fluctuation than *B. a. americanus*. The latter inhabits the mesic Great Lake–St. Lawrence, Acadian and Eastern Deciduous forests over much of its range. Both occur widely in the Boreal Forest in the northern portions of their ranges, and *B. a. americanus* continues into the Boreal–Tundra transition. Harper (1956, 1963) has pointed out that the northern limit of each is reached south of the limit of permafrost.

The appearance of the cranial crests is the most striking difference between *B. a. hemiophrys* and *B. a. americanus*. This difference may be related to selection pressures arising from differences in soil type and aridity in the respective environments of the two forms. Although experimental evidence is lacking, the cranial crests may function as the protection for the eyes, since the latter can be withdrawn into the head below the level of the crests. The solid or grooved boss formed by the crests of *B. a. hemiophrys* may be of advantage in emerging from the heavy prairie soils found over much of its range. Similar thickening, or thickening and filling in of the crests is found in the closely related *B. w. woodhousei*, the more distantly related *B. cognatus*, and in the Plains Spadefoot, *Scaphiopus bombifrons*, which belongs to a separate family. All occur in prairie habitats over much or all of their ranges. In contrast, the eastern forest-dwelling *B. a. americanus* has well-separated, relatively narrow crests (though they may be pronounced posteriorly) as does the eastern *B. w. fowleri*. The Eastern Spadefoot, *Scaphiopus holbrooki*, which typically occurs on light sandy soils, lacks a boss. The toad of the northern forests of western North America, *B. boreas*, either lacks cranial crests, or has only weak, low traces of them.

The relatively shorter tarsus of *B. a. hemiophrys* would result in shorter muscles in this portion of the leg, which may provide additional strength at the expense of some agility compared with *B. a. americanus*. This could be an advantage in digging in heavier prairie soils and for digging to greater depths to avoid aridity and frost. The relatively large spade in *B. a. hemiophrys* would also be an adaptation for more efficient digging in more difficult conditions. *B. cognatus* has a large spade as well, whereas this structure is relatively narrower in *B. a. americanus* and much narrower in *B. boreas*. The parallel reduction of the inner metatarsal tubercle that seems coincident with a large spade is noteworthy as the spadefoots (*Scaphiopus*), which are primarily arid-adapted, lack this tubercle entirely.

Tihen (1962a) postulated that the development of metatarsal spade and heavier limbs is associated with burrowing ability as an adaptation to arid conditions in the "*americanus*" group in which he includes *B. cognatus*.

Martin (1973) found the tibiofibula and femur of *B. a. hemiophrys* to be shorter and broader than all other members of the *B. americanus* group and the humerus shorter than it is in all but *B. woodhousei*. He suggested these proportions were due to selective pressures for efficient burrowing apparatus "in the cold environment" of this species.

Other characters, such as the nostril separation, head width and parotoid gland separation are related to a wider, stockier build in *B. a. hemiophrys* and *B. cognatus* compared with the forest-dwelling forms.

If *B. a. americanus*, exclusive of its northernmost populations, is compared with *B. a. hemiophrys*, the latter has larger dorsal blotches containing smaller and more numerous warts. *B. cognatus* shows a more extreme trend in this direction. However, northern *B. a. americanus* populations have large blotches and generally more warts per blotch. These northern toads may be more diurnal because of longer day length and cooler nights and occur in more open habitats than more southerly *B. a. americanus*. Larger blotches may provide a more contrasted pattern and better camouflage for a toad in open habitats. The more subdued colours in *B. a. hemiophrys*, where grey coloration is common basic background, is evident also in *B. cognatus*. Both lack the deep browns or reds that are evident in some *B. a. americanus* and *B. boreas*, and reach extreme

development in northern populations of the former. Nevo (1973) in a study of colur polymorphism in cricket frogs (*Acris*) found the frequency of a grey morph increased, and red and green morphs decreased, along increasingly arid habitat gradients.

While there is general agreement that *B. a. americanus* is a forest toad, some midwestern populations may show prairie adaptations. Smith (1961) designated it as a "prairie toad" in northern Illinois. However, he also expressed reservations about the equivalence of these populations to *B. a. americanus* Holbrook. Garman (1892) noted that a *B. americanus* he examined from Illinois had the cranial crests bridged posteriorly but did not indicate where in the State it had been collected. Smith (1961) regarded *B. a. charlesmithi* of southern Illinois as a forest toad. The variation and ecology of toads in this and adjacent states deserves further attention, particularly as the post-glacial prairie peninsula once extended over the northern portion of Illinois.

3. Breeding Call Analysis and Comparisons

Blair (1957a) has previously pointed out the distinctions and resemblances between the calls of *B. a. americanus* and *B. a. hemiophrys* and this comparison led him to postulate their close relationship. Henrich (1968) did not have data available for his comparison of these taxa.

The samples for call analysis were restricted to a much smaller geographic area than those available for morphological analysis. Only those tapes recorded during 1969–1970 had body temperatures of calling toads recorded. Zweifel (1968) has stressed the importance of using actual body temperature in analysis of call parameters in preference to the air or water temperatures in the vicinity of a recorded animal. The body temperature of a toad may be close or identical to either that of the air or water in its vicinity. However, body temperature can also have an intermediate value because of recent movement from one medium to the other, and even adjacent toads may have different body temperatures.

The results obtained in this study of the relative effects of temperature and snout-vent length on the call parameters (pulse rate, dominant frequency and duration) are in general agreement with those of Zweifel (1968) who compared samples of 50 *B. a. americanus* from two populations in New Jersey and 63 *B. woodhousei fowleri* from New Jersey and New York populations. Zweifel found a strong positive correlation between temperature and pulse rate in both species and a negative correlation similar to the one reported here for temperature and call length. He did find temperature and dominant frequency to be correlated for Northville, New Jersey, *B. a. americanus* ($r = +0.38$, $P < 0.05$) and this is quite similar to the *B. a. americanus* result in this study ($r = +0.416$, $P < 0.01$), but his Hayworth, N.J., sample showed a weak negative correlation which was not significant ($r = -0.08$, $P > 0.1$).

Zweifel's comparisons of body size with pulse rate, call length and dominant frequency for *B. a. americanus* are similar to the results of this study in that he did not find any significant correlation. His results for *B. w. fowleri* in comparisons of body size, however, gave a significant correlation ($+0.47$, $P = 0.001$) with call length in the Hayworth sample and correlations with dominant frequency of -0.49, ($P < 0.1 > 0.05$) in a Long Island sample and -0.59 ($P < 0.001$) in the one from Hayworth.

A comparison of Zweifel's values for the two most highly correlated variables, pulse rate and temperature, for his two *B. a. americanus* and two *B. w. fowleri* samples with samples analysed in this study of *B. a. americanus* and *B. a. hemiophrys* shows the similarity of the New Jersey and Manitoba–western Ontario populations of *B. a. americanus*. As well, it indicates that *B. a. hemiophrys* occupies an intermediate position between *B. a. americanus* and *B. w. fowleri* (Figure 38).

The separation of taxa based on call and associated variables is equally as effective as the separation on strictly morphological variation. Intermediate populations show the wide variability which is typical of the morphological analysis. However, in the call discrimination the body temperature of the toad has made a contribution, apparently because of the relatively fewer *B. a. hemiophrys* recorded at low readings.

The comparison of the discriminant scores for call and morphology indicates that call variation does not follow morphological variation within either taxon, but that in the intermediate populations there is a tendency for call type to correlate with morphological type. Within the contact zone *B. a. hemiophrys*-like toads morphologically tend to have *B. a. hemiophrys*-like calls and *B. a. americanus*-like morphologically tend to have *B. a. americanus*-like calls. The comparison of the

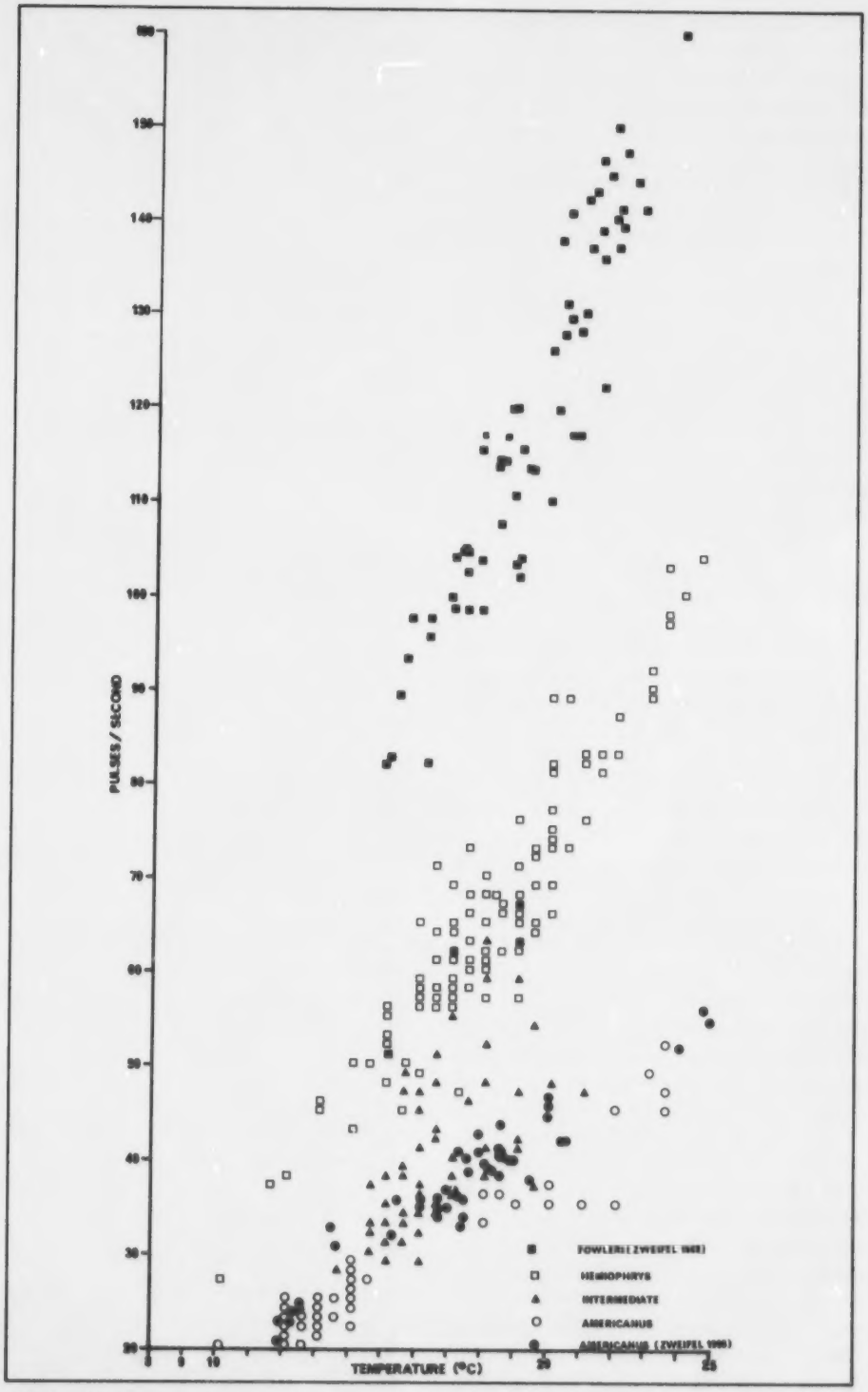


Figure 38. Relationship between pulse rate and temperature in three samples of *Bufo* from this study, and two *B. a. americanus* and two *B. w. fowleri* populations from northern New Jersey and Long Island, New York (data from Zweifel 1968: Figure 8).

total pooled sample shows the same correlation of call and morphology between taxa (Table 8).

4. Crossbreeding Experiments

The most important result of these crosses is that all combinations can produce metamorphosing individuals. This supports the morphological evidence that natural intermediates are freely produced. Unfortunately, the experiments were terminated at transformation. However, the success of toads from intermediate localities, as well as those from the most separated localities, indicates that no absolute fertility barriers exist. The differences in results in some of the replicate crosses and the unequal number of replicates makes any conclusion based on relative success between combinations unreliable.

Previous hybridization experiments summarized in Blair (1972) involving *B. a. hemiophrys* and *B. a. americanus* have also demonstrated their ability to produce metamorphosing offspring. Porter (1968) demonstrated fertility between individuals from the Wyoming relict population of *B. a. hemiophrys* and those from North Dakota and Manitoba.

Blair (1972: Appendix H) reported two artificial crosses involving a female *B. a. americanus* and a male *B. a. hemiophrys* in which 85.2% and 34.9% of the resulting larvae metamorphosed. Three crosses involving a *B. a. hemiophrys* female and an *B. a. americanus* male gave 64.3, 93.9 and 43.5% fertilization of eggs. In two of the crosses 37.8% and 86.9% of the fertilized eggs hatched and 42.0 % and 29.4% of the larvae reached metamorphosis.

5. Taxonomic Conclusions

a) Comparisons of *B. a. hemiophrys* and *B. a. americanus* with other *Bufo* in northern North America

(i) *B. a. hemiophrys–B. boreas:* In southern Alberta, *B. boreas* is restricted to the Rocky Mountains and their foothills, whereas *B. a. hemiophrys* occurs in Grassland and Aspen Parkland regions to the edge of the foothills. To date, no contact has been found in this region (unpublished results of herpetofaunal surveys of Canadian Prairie Provinces). However, north of Edmonton, *B. boreas* occurs west of the foothills in the Boreal Forest region and has a narrow area of sympatry with *B. a. hemiophrys*. To the northwest, only *B. boreas* occurs in Aspen Parkland islands of the Peace River region, an area where, by habitat, *B. a. hemiophrys* would be expected.

Eastern Alberta *B. boreas*, although larger than *B. a. hemiophrys* at these localities, are markedly smaller than typical *B. boreas* from British Columbia (unpublished NMC data). Also, although it has been often asserted that *B. boreas* lacks a true breeding call (Blair 1972 and elsewhere), populations in eastern Alberta have a distinct call, which has a low dominant frequency and is very slowly pulsed. It has been noted at Banff, High River, and in the overlap zone with *B. a. hemiophrys* (unpublished NMNS data). Although very distinct from the higher, rapidly pulsed call of *B. a. hemiophrys*, the frequency of mismatings in mixed choruses throws doubt on its complete effectiveness as an isolating mechanism within a common breeding pond. Different calls may, however, serve to differentially attract females and males when breeding ponds are distinct.

Blair (1972: Appendix H) reported two artificial crosses between female *B. a. hemiophrys* and male *B. boreas* in which 41.7% and 79.7% of the eggs were fertilized and 77.4% and 3.2% of the larvae metamorphosed. Only one cross involving a female *B. boreas* and a male *B. a. hemiophrys* was reported and this resulted in 98.2% fertilization and 81.8% hatching but none of the resulting larvae reached metamorphosis. No naturally occurring hybrids have been previously reported between these taxa.

The *B. boreas–B. a. hemiophrys* overlap without loss of distinctness and only rare survival of natural hybrids provides an interesting contrast to the *B. a. americanus–hemiophrys* interactions. However, the narrowness of the overlap, the apparent similarity in breeding time and site, and the closeness in body size may be indications that these two forms have not diverged enough to partition an area between them.

(ii) *B. a. hemiophrys–B. cognatus:* *B. a. hemiophrys* and *B. cognatus* are sympatric in the shortgrass prairie or southern Alberta and Saskatchewan and the adjacent Dakotas and western Minnesota. *B. cognatus* ranges south into Mexico (Stebbins 1966), is much more grassland adapted than *B. a. hemiophrys* and their sympatric area, which is much broader than that of *B. a. hemiophrys* and *B. boreas*, represents the area where *B. cognatus* reaches its northern limit and *B. a. hemiophrys*, with the exception of the

Wyoming relict, reaches its southern limit. In Minnesota, a natural hybrid between *B. cognatus* and *B. a. hemiophrys* has been reported (Brown and Ewert 1971).

B. cognatus has a very long distinctive call resembling the sound of a pneumatic hammer (Conant 1975). This call has tremendous carrying capacity and is strikingly distinctive from calls of the *B. americanus* group. The vocalization of males in this chorus could be heard at least 0.5 mile [0.8 km] over the prairie from the road where we had stopped our vehicle. *B. a. hemiophrys* was not heard in this din until we were much closer to the pond.

Blair (1972b: Appendix H) has recorded one artificial cross of a female *B. a. hemiophrys* and a male *B. cognatus* where 68.0% of the eggs were fertilized, 73.5% of those fertilized hatched, but only 3.5% of the larvae metamorphosed. In addition, in one cross of a *B. cognatus* female and a *B. a. hemiophrys* male 33.3% of the eggs were fertilized but none hatched, all stopping in gastrula or neurula stage.

(iii) *B. a. hemiophrys–B. w. woodhousei* and *B. a. americanus–B. w. fowleri:* The subspecies *B. w. woodhousei* is not known to extend into Canada and in the northern United States. Its range as currently understood is allopatric to that of *B. a. hemiophrys*.

Blair (1972: Appendix H) reported two crosses using a female *B. a. hemiophrys* and a male *B. w. woodhousei*. One of these resulted in 97.0% fertility, with 83.6% of the fertilized eggs hatching and 26.6% of the larvae reaching metamorphosis. For the other the only data given are that seven larvae were obtained and 85.6% (or six of them) metamorphosed. In two crosses between a *B. woodhousei* female and a *B. a. hemiophrys* male given in the same report, 100% were fertilized and 24.4% of 600 larvae metamorphosed in the first and 31.2% of 279 larvae metamorphosed in the second. These values for hatching success are only marginally lower than those obtained with crosses between *B. a. hemiophrys* and *B. a. americanus*.

The subspecies *B. w. woodhousei* has prominent, usually somewhat parallel cranial crests, few warts per dorsal blotch and generally has an unspotted venter. Blair (1972) mentions that Great Basin populations are smaller and may have a cranial boss, but there is no published comparison of this variant with prominently bossed *B. a. hemiophrys*. *B. w. woodhousei* occurs primarily in the great plains of the central United States, often in river valleys, and is sympatric over much of its range with *B. cognatus*, which tends to occupy the upland prairies (Timken and Dunlap 1965).

The other northern subspecies of *B. woodhousei, B. w. fowleri*, is eastern in distribution and largely sympatric with *B. a. americanus*. It is smaller than *B. w. woodhousei* and generally has more warts per dorsal blotch. Its intergradation with *B. w. woodhousei* has been documented by Mecham (1962). Underhill (1961b) has compared morphological variation between *B. a. hemiophrys* and *B. w. woodhousei* in South Dakota but his sample of the former was probably contaminated by introgression from *B. a. americanus* (cf. localities given by Underhill 1961 and Henrich 1968).

The call of *B. w. fowleri* has a faster pulse rate than that of *B. a. hemiophrys* (Figure 38). It is interesting to note that, while *B. w. fowleri* is intermediate between *B. a. hemiophrys* and *B. a. americanus* morphologically in the characters studied here, *B. a. hemiophrys* is the intermediate with respect to this call variable. The *B. a. hemiophrys* samples geographically closest to the northern limit of *B. w. woodhousei* (those from the prairie grassland of southern Alberta and southwestern Saskatchewan) have low mean discriminant scores (-30.8 to -26.9). These give no indication of introgression from interbreeding with *B. w. woodhousei* further south as, if this was affecting southern *B. a. hemiophrys* scores it would presumably raise them. Another indication that interbreeding of *B. w. woodhousei* and *B. a. hemiophrys* is not widespread is the survival of the Wyoming disjunct of *B. a. hemiophrys* within *B. w. woodhousei* range. However, the narrowness of the contact zones between other *Bufo* taxa where complete interbreeding can occur leads one to be cautious in drawing any but tentative conclusions until adjacent populations can be analysed. The possibility exists that introgression from *B. w. woodhousei* had some effect on the scores obtained by Henrich (1968) for his 160-mile [257.6 km] intergradation zone between *B. a. hemiophrys* and *B. a. americanus* in South Dakota. Much of this zone is a southern extension of *B. a. hemiophrys* which is not only adjacent to *B. a. americanus* on its east but also to *B. w. woodhousei* on its west. Comparison with *B. w. woodhousei* was not made by Henrich. *Bufo hemiophrys* was considered a subspecies of *B.*

woodhousei by Schmidt (1953) but Blair (1957a) presented convincing evidence for its closer relationship to *B. americanus*.

Similarly, the mean discriminant value for *B. a. americanus* samples adjacent to the range of *B. w. fowleri* (+33.8 at Rondeau and +35.3 at Point Pelee) in high, perhaps even indicating divergence (character displacement of Brown and Wilson 1956) between these forms in adjacent localities. Elsewhere in a wide area of geographic sympatry the two taxa are known to hybridize (e.g. Zweifel 1958), and selection against hybrids may be an important factor here.

b) Status of *B. americanus copei* and *B. hemiophrys baxteri*

(i) *B. a. copei*: Northeastern *B. americanus* Populations: The validity of the northeastern *Bufo americanus copei* (Yarrow and Henshaw 1878) is open to question. Unmistakably, there are populations of brightly coloured *B. americanus* with contrasted patterns accentuating reds and oranges in the northern portion of its range. These populations have also been characterized as having heavily pigmented venters and shorter hind legs. Such characters formed the basis for the initial recognition as *B. copei* as a distinctive form, and for Gaige (1932) to have resurrected it from synonomy as *B. a. copei*. It was first described from James Bay material, but subsequently Trapido and Clausen (1938), Grant (1941), Vladykov (1941), Netting and Goin (1946), Backus (1954) and Harper (1956) extended its range across northern Quebec. Schueler (1973) reaffirmed the distinctive coloration of James Bay toads. Bleakney (1952) applied the name to Nova Scotia populations outside the Annapolis Valley which had shorter tibias and more heavily pigmented venters. Subsequently, however, Bleakney (1958) did not recognize subspecies within *B. americanus* in eastern Canada. Most recently, Ashton, Guttman and Buckley (1973) extended the range of *B. a. copei* south over much of central Ontario and Quebec. However, *B. a. copei* characters are not pronounced in all northern individuals (Cook 1964, 1968). Earlier Logier (1952) and Logier and Toner (1955, 1961) presented a discussion of the validity of the race in which they pointed out the intermediate nature of supposedly diagnostic characters across a wide geographic area. Of all these papers, only Logier (1952) presented any statistical comparison of *B. a. copei* and *B. a. americanus* populations, but only relative leg length was treated. In the present study neither tibia nor tarsus length appear to be useful in defining a distinct northern race within *B. a. americanus*.

Two measurements of variation in the present analysis which do have bearing on the status of *B. a. copei* are the length of the dorsal spot and the ventral pigmentation score. Particularly high mean values for these characters are present in northern population samples: Whitetop, Moosonee, Lake Attila, Sept Îles, Mile 134 [Kilometre 215.7], and Routhierville. To the south there is a wide zone of intermediates until the "typical" (southern) *B. a. americanus* with smaller dorsal spots and a less heavily pigmented venter is found. The variation in coloration seems to parallel these characters with the most brightly coloured toads in the north. The wide dorsal stripe and more contrasted pattern mentioned by most observers recognizing *B. a. copei* are also evident in the material examined here and specimens with large dorsal blotches generally have these features as well. The dorsal "stripe" cited by previous authors seems to be an area largely defined by the dorsal blotches, and may therefore be highly irregular in width, varying with evenness of blotch edges and difficult to measure objectively. A "trace" mid-dorsal stripe, which is very narrow with parallel edges, can also be seen on many specimens and is somewhat paler than the broad stripe. This narrow stripe is widespread in *B. americanus* group toads.

Northern *B. americanus* are apparently often strongly diurnal in habit (personal communications: Ross MacCulloch, northern Quebec; F.W. Schueler, James Bay) in contrast to the generally nocturnal or crepuscular behaviour, especially of adults, further south (FitzGerald and Bider 1974: 65 miles [104.7 km] NW of Montréal, Quebec). Diurnal activity is to be expected in the north because of long day length and cool evening temperatures during the short summer. The bright colours and highly contrasted pattern of northern populations may be of selective advantage during diurnal activity because of the disruptive visual effect of such contrasts.

Because of the wide area which is transitional from north to south, there seems little justification in distinguishing northern populations as a distinct subspecies. As Logier and Toner (1955, 1961) have pointed out, the intergrade zone would be wider than the range of the northern race. Ashton, Guttman and Buckley (1973) attempted

to resolve this difficulty by redefining *B. a. copei* to include most of these intermediate populations but failed to provide evidence that this enlarged concept of *B. a. copei* could be adequately distinguished from southern *B. a. americanus*. Guttman (1975) failed to find distinctive isozyme distributions between *B. a. copei* and *B. a. americanus*. The reduction in blotch size and ventral pigmentation in *B. americanus* is toward its southern area of sympatry with *B. w. fowleri*. It is suggested here that the transition between "*copei*" and southern *B. americanus* is due in part to different selective pressures on extreme northern and southern populations and a cline through the intervening area.

(ii) *B. h. baxteri*: the Wyoming Disjunct of *B. a. hemiophrys*: *B. h. baxteri* was named by Porter (1968) from a comparison of 34 Wyoming specimens and an equal number from a composite sample drawn from North and South Dakota and Manitoba. The differences used to define the subspecies were mainly statistical comparisons of selected morphological characters.

He concluded that Wyoming toads tended to be smaller, with shorter radio-ulna and tibio-fibula measurements, narrower heads, a more prominent boss and a narrower mid-dorsal stripe. Ulna length, boss height and mid-dorsal stripe were excluded from the present study. However, measurements of snout-vent length, tibia length and head width (remeasured on most of Porter's sample) do not distinguish Wyoming populations when considered against the variation in populations from throughout the range *B. a. hemiophrys*. The relatively small differences found by Porter in the characters not measured here would probably not validate the distinctiveness of the Wyoming *B. a. hemiophrys*. The discriminant score mean for the Wyoming population is well within the range of score means over the rest of the geographic range of *B. a. hemiophrys*.

Criteria for subspecies vary widely between workers, but commonly a significant proportion of individuals within a geographic area have to be distinguishable from all other individuals in other areas to warrant taxonomic recognition (the so-called 75% rule). Smith (1974) has argued that an "abrupt geographical step" in the variation of at least one character should be present to warrant subspecies recognition. *B. h. baxteri* does not appear to meet either criterion for a subspecies. A disjunct distribution is not generally recognized as sufficient cause for erecting of a taxon unless distinguishing morphological or other characters can be clearly defined (Mayr 1963). The overemphasis of the significance of its geographic isolation even led Packard (1972) to recommend the recognition of *Bufo baxteri* as a full species. However, Porter (1968) presented evidence that Wyoming toads were successful in crossing with Manitoba and Dakota toads under laboratory conditions. There is no reason to suppose they would not interbreed freely in nature if re-expansion of range brought the Wyoming population into contact with any other *B. a. hemiophrys* populations. Porter (1968) found only slight difference in the call of the Wyoming population. As he did not record toad body temperatures exact comparison of his data with the variation presented here could not be attempted, but I do not believe that the differences appear significant.

c. The Status of *B. a. americanus* and *B. a. hemiophrys*

The contrast between results in the analysis of southeastern Manitoba samples compared with the reference samples from throughout the range of *B. a. hemiophrys* and northern *B. a. americanus* is evident. Clearly the distinction between the morphologically "good species", "*B. americanus*" and "*B. hemiophrys*", disintegrates biologically across this zone, and extensive interbreeding and backcrossing are freely occurring in the apparent absence of strong isolating mechanisms. The narrowness of the contact zone between the taxa is emphasized by both morphological and call analysis. Its limited extent may be a function of the relative abruptness of the vegetation ecotone where it occurs. It may also be affected by the distance moved by individual toads in their lifetime.

This situation may be designated allopatric hybridization in the sense of Woodruff (1973). Both parental forms are distinct on either side of a zone populated by intermediate animals but pure individuals of either parent are not clearly distinguishable within the intermediate zone. This term contrasts with Woodruff's parapatric hybridization, where both parental forms are identifiable in an intermediate zone containing hybrids, and sympatric hybridization, where the ranges of the parental forms may extensively overlap and hybrids are produced in varying

numbers. These interactions represent various gradations between the relatively free and complete gene flow generally assumed to occur within a species and the absolute reproductive barriers (no gene exchange) between indisputably good species. The various natural interactions which demonstrate some, but incomplete, isolation between populations have been designated as semispecies, allospecies or incipient species as extensively reviewed by Amadon and Short (1976).

Mayr (1963, 1969, 1970) clearly regards cases where extensive interbreeding occurs between two essentially allopatric populations as evidence of their lack of reproductive isolation and concludes that such populations belong to the same species. Rising (1970) reviewed some of the inconsistent treatment of a selection of such taxa by many workers. Bigelow (1965) argued that reproductive isolation rather than interbreeding is the essential part of Mayr's definition and that the former could exist and be maintained even if interbreeding produces a range of recombinations of the parental genotypes in a hybrid zone. Although such forms may have no barriers to interbreeding where they meet, he reasoned, strong selection on either side of the hybrid zone which eliminates variants carrying genes of the other form would produce effective isolation. Short (1969) suggested that Bigelow ignored the differential action of natural selection on characteristics. One or a few genes from one differentiated population may be incorporated into another due to interbreeding and, as selection is unlikely to be so severe that some introgression is not occurring, complete reproductive isolation is not likely to be maintained by selection where there are no barriers to free interbreeding. Only the sympatric occurrence of the two forms can be taken as an indication of reproductive barriers. Mayr (1963) largely discounted introgression as of any consequence between species in animals, as he regarded species' gene pools to be internally cohesive and coadapted that they are largely resistant to incorporating genes from other gene pools.

Sibley (1969) has provided a framework for taxonomic judgement of the varying grades of interbreeding situations. Evidence, or lack or it, for some degree of reduction in interbreeding as shown by at least some degree of sympatry is judged to be of paramount importance. The natural occurrence of some hybrid individuals or some geographic areas where complete interbreeding occurs (including isolated instances of local hybrid swarms) is weighed against the geographic extent or numerical preponderence of "pure" forms or populations in contact. If contact without extensive interbreeding predominates, the taxa have species status. But, if in the majority of contacts, interbreeding between distinct taxa produces a population in which all or nearly all individuals have some degree of intermediate characteristics the taxa are regarded as subspecies. Their gene pools are obviously not isolated.

Schueler and Rising (1976) provided a thoughtful analysis of cases where "hybridization" could be recognized, stressing the importance of mean, variance and modality of discriminant analysis scores. Although they would regard results such as obtained here as indicating hybridization, it is unclear if they would consider species status a mandatory consequence.

In the present case, interbreeding occurs between *B. a. americanus* and *B. a. hemiophrys* throughout their contact zone in eastern Manitoba. Henrich (1968) has clearly demonstrated the same situation at the opposite end of their contact, in South Dakota. Although Henrich's zone as presented is apparently much wider, his measure (a coefficient of similarity) may not be as precise for delimiting the contact zone as the discriminant function, and therefore the results of the two studies are not exactly comparable. His sampling area ran in a NW-SE direction, and may be cutting the contact area on a diagonal, rather than at right angles, thus not evaluating the true width of contact. A similar effect is noticeable in the "southern" transect presented here. It has been suggested earlier that analysis of the South Dakota contact should also consider possible introgression from *B. w. woodhousei* on the west, which could introduce additional variability, and perhaps broaden the apparent contact area.

Minnesota, in contrast to the northern and southern contact in Manitoba and South Dakota, respectively, has not received comparable analysis. That the contact zone there is narrow is apparent from statements by Breckenridge (1944), Blair (1957a), and Tester, Parker and Siniff (1965), who had no difficulty in assigning collections to one taxon or the other, and doubted any range overlap existed. Henrich included a few Minnesota specimens in his analysis and found evidence of intermediacy of characters. Guttman (1969) found

some transferrins with identical mobilities in Minnesota *B. a. hemiophrys* and *B. a. americanus*.

This evidence indicates a continuous interbreeding zone between the southern end of Lake Manitoba through Minnesota to northern South Dakota wherever *B. a. hemiophrys* has come in contact with *B. a. americanus*. The contact is centred on the ecotone between Great Lakes and Deciduous Forest with Parkland and Prairie (this study; Henrich 1968).

No evidence exists of primary isolating mechanisms (inability to physically mate or absolute sterility of hybrids) or strongly developed secondary isolating mechanisms (ecological or temporal) (Mayr 1963).

The difference in calls may, however, be a weakly effective behavioural isolating mechanism. Licht (1976) in a study of *B. a. americanus* has suggested that female toads select mates, and has presented some observational evidence. He hypothesizes that call variables of vocal males allow females to select a male within a narrow size range. The evidence for call variables following precisely enough with male snout-vent length within a single taxon to allow such discrimination is weak (this study; Zweifel 1968). If the choruses in the intermediate zone consisted of clearly definable parental forms and hybrids, such discrimination by females might be reasonably postulated. However this is not the case. The majority of toads in the contact zone are intermediate in both call and morphology, and no clear assortment into parental types and hybrids is evident. Mate selection by females, if it occurs here, may be effective at the edges of the contact zone in allowing a female to select a "pure" male, or chorus, instead of a hybrid individual or chorus of hybrids. This may increase in effectiveness with distance to the east and west of the zone. It is interesting to note in this regard that the *B. a. hemiophrys* males west of the contact zone are the smallest that occur (both in range and mean). Some character displacement (Brown and Wilson 1956) in size may presently be occurring in *B. a. hemiophrys*. This may account, in part, for the reduced extent of apparent introgression of *B. a. americanus* genes into *B. a. hemiophrys* populations.

It seems reasonable to assume that if *B. a. hemiophrys* characters are adaptations for relatively more arid environments, and if selection is most severe on toads in arid habitats because of recurrent drought then, "mesic-adapted" genes may be less able to penetrate westward than "arid-adapted" genes can penetrate eastward. The geographic distribution of discriminate scores suggests that this could be true. Higher variances in discriminate scores on the eastern side of the contact zone, and the possible character displacement in size of *B. a. hemiophrys* to the west support the possibility of more restricted gene flow to the west than to the east of the contact zone. Tester, Parker and Siniff (1965) concluded, from behavioural studies, that *B. a. americanus* is less well adapted to avoid heat stress in open areas than *B. a. hemiophrys*. This could impose further restriction on the westward flow of *B. a. americanus* genes.

In summation, although *B. a. americanus* and *B. a. hemiophrys* are clearly as distinct as good species in morphology and call where allopatric, the lack of any recognizable sympatry between pure forms of each indicates a lack of isolation mechanisms where they contact each other. They cannot be considered as having isolated gene pools and therefore should be treated as subspecies (Sibley 1961; Short 1969; Mayr 1963; Woodruff 1973; Amadon and Short 1976).

The morphological distinctness of *B. a. americanus* and *B. a. hemiophrys* and the high variability of hybrid populations indicates that their respective gene pools have incompatabilities and are perhaps somewhat differentiated. Amadon and Short (1976) have used the term megasubspecies for well-marked subspecies known, or judged to be, approaching the status of a species. In trinomials designating a megasubspecies the species name is placed in parentheses, and the taxa here should be written:

Bufo (americanus) americanus
Bufo (americanus) hemiophrys

This notation complements Amadon's (1966, 1968) suggestion that allospecies should be listed with the superspecies of which they are a member placed in brackets. The distinction between allospecies and megasubspecies is developed from the discussion given earlier by Short (1969). When the designated forms contact a closely related form a predominance of allopatric hybridization in the sense of Woodruff (1973) indicates megasubspecies and a predominance of parapatric or sympatric hybridization indicates allospecies. The utility of this notation will be mainly in zoogeographic studies to clarify the relationships of taxa where comparison of faunas of distinct areas is made. For most purposes the brackets may be omitted.

6. Zoogeographic Significance of the Eastern Manitoba Transition Zone

The significance of location of the *B. a. americanus–hemiophrys* transition and its origin can be related to the general herpetofauna distribution patterns of northern North America.

Eastern Manitoba is the centre of a transition between eastern and central herpetofaunas (Cook 1974). The *B. a. americanus–hemiophrys* contact line defined here also is the approximate western limit in southeastern Manitoba for at least two eastern species, the Blue-spotted Salamander, *Ambystoma laterale*, and the Spring Peeper, *Hyla crucifer*. It is the western limit for at least one central species, the Plains Garter Snake, *Thamnophis radix*. In addition, the wide intergrade zone between the central Red-sided Garter Snake, *Thamnophis sirtalis parietalis*, and the Eastern Garter Snake, *T. s. sirtalis*, which extends from extreme western Ontario to central Manitoba, is approximately centred on this line (Cook, unpublished data) and part of the overlap between the Gray Treefrogs *Hyla chrysoscelis* (western) and *Hyla versicolor* (eastern) in Manitoba occurs here (Stewart and Cook 1970, and unpublished data). This contact zone may be regarded as a zoogeographic suture zone in the sense of Remington (1968), a region where two faunas thought to be previously separated have re-established contact resulting in a variety of interactions. Because the northern faunas are poor in species diversity, the number of interactions are few. As pointed out above, they vary from the zone of sympatry of apparently good species in *Hyla versicolor* and *H. chrysoscelis* to the wide area of subspecies intergradation between *T. s. parietalis* and *T. s. sirtalis*. The *B. a. americanus–hemiophrys* interaction is intermediate between these extremes, showing a sharply demarcated contact with limited introgression.

Western limits for widely distributed eastern amphibians and reptiles correspond to gradually increasing aridity from east to west (Cook 1975; see also Figure 77, p. 295, *in* Bryson and Wendland 1967), as do, in reverse, eastern limits for many central forms. A marked change in relative aridity in the Manitoba lowlands is indicated by the sharp break between largely coniferous forest and aspen parkland. The *B. a. americanus–hemiophrys* peak interbreeding seems centred in this area.

The central herpetofauna as defined by Cook (1974) is bounded by this eastern Manitoba suture-zone in the east and by the foothills of the Rocky Mountains in the west. The majority of amphibian and reptile species which range north into the Boreal Forest areas in each of the eastern, central, and western regions between these boundaries are specifically or subspecifically unique to each region. The transcontinental Boreal Herpetofaunal Zone postulated by Savage (1960) extending from Alaska to the Atlantic coast, seems based primarily on one species, the Wood Frog, *Rana sylvatica*, which is the sole taxon which ranges widely across all three regions. However, the Wood Frog is not restricted to Savage's supposed Boreal Zone but occurs far south of it in the east continuously and in the west as disjunct populations. In the central region, all Boreal species (*Rana sylvatica*; the Leopard Frog, *Rana pipiens*; the Boreal Chorus Frog, *Pseudacris triseriata maculata*; *B. (a.) hemiophrys* and *T. s. parietalis*) also occur throughout the adjacent Aspen Parkland. In the east, three wide-ranging Boreal species, *Ambystoma laterale, Hyla crucifer*, and the Mink Frog, *Rana septentrionalis*, reach their western limit in southeastern Manitoba at or near the Aspen Parkland boundary. None invades the Aspen Parkland. Two others, *B. (a.) americanus* and *T. s. sirtalis*, are replaced over this boundary by central subspecies. None of these five eastern forms occurs in the central Boreal Forest although suitable habitat appears to exist for them there. However, all central Boreal Forest species either show some range extension or genetic influence to the east. In *R. pipiens* no differences between central and eastern northern populations have been defined. In *R. sylvatica* a striped morph, common in central populations, shows a decreasing abundance from west to east in northern Canada. The number of individuals possessing a stripe shows its most rapid drop south and east of southern James Bay (Schueler and Cook 1980). *Pseudacris t. maculata* ranges east to Moosonee on southern James Bay. South of the Great Lakes it is replaced by an eastern subspecies, the Western Chorus Frog, *P. t. triseriata*. These forms do not make contact in Ontario (Cook 1964b; Weller and Palermo 1976; and unpublished observations) but do southwest of Lake Superior (Conant 1975). This study presents evidence of introgression in *Bufo a. americanus* from the central form *(B. a. hemiophrys)* east to James Bay in western Ontario. This area corresponds to the eastern range of *Pseudacris t. maculata*. The influence of *T. s.*

parietalis into *T. s. sirtalis* populations is not known to be as extensive, apparently reaching its eastern limit in extreme western Ontario. These distributions of species and variations may indicate past isolation of central and eastern herpetofaunas, followed by the re-establishment of contact and subsequent eastward dispersal of some forms and gene flow from others primarily from the central region into the eastern region.

Most of the regions discussed here were covered by glacial ice during at least four successive continental glaciations (Flint 1971) each of which would have obliterated northern faunas and floras over their present ranges. It is generally assumed that much of the present northern fauna and flora was displaced southward in response to changing climatic conditions south of glaciation and survived south of the ice sheet in "gross refugia" (Lindroth 1969). The most recent continental glaciation, the Wisconsin, reached its maximum extent about 18,000 years ago (Prest *et al.* 1969). Bryson and Wendland (1967), Flint (1971) and Terasmae (1973) present maps reconstructing climate and vegetation during the Wisconsin maximum which depict the entire glacial margin bordered to the south by tundra and boreal forest. However, recent evidence indicates the possibility of a region in Nebraska during peak glaciation of drifting sand dunes which may have been treeless or even vegetationless (Wright 1971). If the area between these dunes and the glacier margin was occupied only by tundra, the Boreal Forest would be broken into eastern and central segments, assuming the southern edge of the dune areas was occupied by prairie vegetation. (Mengel (1970) invokes this possible break in discussing zoogeography of bird taxa.) Present central Boreal forms show a gradation of southern limits from *Rana sylvatica* which does not penetrate grassland habitats (Cook 1965b) to *Pseudacris triseriata maculata* which is well adapted to northern grassland but is replaced by related species in the southern prairies (Conant 1975). All would be excluded from a severely arid barrier. If the Boreal Forest was broken in central North America, central Boreal forms would be isolated along the eastern side of the Rocky Mountains and adjacent areas. *Rana sylvatica* and *B. (a.) hemiophrys* now have disjunct populations in southeastern Wyoming and the present ranges of *Rana pipiens, Pseudacris* and *T. s. parietalis* also include this area (Conant 1975, Stebbins 1966).

Although not a primary barrier, post-glacial Lake Agassiz which covered much of southern Manitoba (Mayer-Oaks 1967) may have had an effect on the present distribution patterns. It has been postulated by Löve (1959) that the vegetation which first covered the lake bed after Lake Agassiz drained was grassland and another grassland interval occurred in the Hypsithermal. During either of these transitional periods, *B. (a.) hemiophrys*, assuming it had reached the area, may have been able to invade farther east than at present. At least some of what has been interpreted here as eastward introgression of *B. a. hemiophrys* genes could be the result of initial *B. a. hemiophrys* invasion of the area, and subsequent swamping by *B. a. americanus* which spread into the area with the invading species from the eastern forest. However, it is unlikely that the postulated post-Agassiz invasion of *B. a. hemiophrys* was extensive enough to explain the far eastern extent (James Bay) of indications of introgression from *B. a. hemiophrys*.

The east-central faunal split may have occurred much earlier than the Wisconsin or more than once through successive Pleistocene glaciations. Tihen (1962b) tentatively identified material from the Kansan glaciation in Kansas as similar to *B. a. hemiophrys*. Tihen (1972) has suggested that *B. hibbardi* from the Miocene-Pliocene boundary and the Middle Pliocene, and *B. rexroadensis* of the Upper Pliocene may represent temporal stages in a single population line leading to *B. woodhousei*. If this interpretation is correct it indicates the comparative antiquity of the *B. americanus* group and the long period available to differentiate distinctive forms. In a survey by Gelbach (1965), the oldest fossil assigned to other members of the *B. americanus* group is the tentative *B. a. hemiophrys* above.

Many cases of interbreeding between morphologically distinctive taxa have been explained in terms of comparatively recent human changes to the environment, particularly through land clearing for agriculture (Mayr 1963). In the present case, the interbreeding sampled is along east-west highways through a relatively well-drained area (Warkentin 1967). Here roadside ditches provide new breeding sites across the transition zone. If call serves to attract females and/or other males to breeding sites (Bogert 1960), then lack of ponds before human alterations at the eastern forest-Aspen Parkland transition could have lessened the

chance of a male coming to a pond in the "wrong" vegetation zone and of a female responding to the "wrong" chorus. East-west highways through the region are a comparatively recent development, and even east-west railways date back only about 100 years. However, the evidence for extensive eastern introgression argues against contact being recent enough to have been man-induced. A test of this hypothesis would be in the relatively unaltered area north of Lake Winnipeg where the nature of contact is yet unknown. *B. a. hemiophrys* and *B. a. americanus* are present in the area, and their ranges are in close proximity. Harper (1963) noted what he recognized as calls of *B. a. hemiophrys* at Wabowden, and Vere Scott (personal communications based on his own observations and those of Leigh M. Nelson and Gordon Shaw) identified *B. a. americanus* from Warren's Landing and William River at the north end of Lake Winnipeg, based largely on the diagnostic cranial crests. Field surveys conducted by F.W. Schueler, R.M. Rankin, F.D. Ross, and S.F. Baird in May and June 1980 at the north end of Lake Winnipeg have confirmed the existence there of a hybrid zone similar to that in southeastern Manitoba. Detailed analysis will be published later.

Endler (1977) has developed a substantial alternative argument to explain what have often, in the past and in this paper, been regarded as points of post-glacial secondary contact between previously geographically separated populations. He shows that, in theory, such sudden morphological changes could be induced by strongly opposing selective pressures greatly reducing gene flow over an abrupt ecological change. The formation of these sharp breaks in variation are postulated to have been possible even in the short period since glaciation without any previous absolute geographic isolation. In retaining a more traditional (zoogeographic) viewpoint here, I have been swayed by the degree of morphological similarity within *B. a. americanus* and *B. a. hemiophrys* populations over their respective ranges, despite the other ecological changes they encounter, as contrasted with the sharp change apparent over the Aspen Parkland–eastern Boreal ecotone in southwestern Manitoba. The more gradual colour and pattern variation exhibited by *B. a. americanus* populations primarily on a north-south axis, discussed earlier, seems, however, to be more in line with Endler's models, and requires no postulation of previous geographic isolation.

7. Taxonomic Significance of the *B. a. americanus–hemiophrys* Relationship with Respect to the Other Nominal Species of the *B. americanus* Group

The *B. americanus* group of Blair (1972) consists of *B. woodhousei*, regarded as an early split from the remaining species, and the largely or completely allopatric *B. terrestris, B. americanus, B. houstonensis, B. hemiophrys* and *B. microscaphus* (Conant 1975; Stebbins 1966). *B. woodhousei* is known to hybridize naturally with all of these taxa except *B. hemiophrys*. A study of the region where contact between *B. woodhousei* and *B. hemiophrys* is probable through South Dakota, North Dakota and Montana is long overdue. *B. terrestris* and *B. americanus* may hybridize along a contact zone but the evidence is disputed (Netting and Goin 1946; Neil 1949). *B. houstonensis* in Texas and *B. microscaphus* in the southwest are disjunct from contact with other taxa in the *B. americanus* subgroup. *B. houstonensis* shows some features of both *B. terrestris* and *B. americanus* (Sanders 1953) but is commonly assumed to be a postglacial isolate of *B. americanus* (Blair 1972). Blair (1972 and earlier papers) suggested an east-west split of a *B. hemiophrys–microscaphus* stock from the eastern *B. americanus* stock. Sanders (1961) postulated hybridization and introgression between *americanus* and an invasion of a broad-skulled toad, possibly of the *B. valliceps* group, as an explanation for the divergent osteological features of *B. terrestris*. A.P. Blair (1955) postulated introgression from a *B. boreas* stock to account for some morphological features of *B. microscaphus*, although this view was not considered by W.F. Blair (1957b). This study shows that hybridization between *B. a. hemiophrys* and *B. boreas* results in a toad with well-separated, low, somewhat *B. a. americanus*-like crests. A previously defined subspecies of *B. woodhousei*, *B. w. veletus* in Texas and Louisiana, is now regarded as the product of hybridization between *B. w. woodhousei*, *B. w. fowleri*, and *B. americanus* (Conant 1975).

W.F. Blair (1972 and earlier papers) has shown that toads of the *B. americanus* group have a marked ability to hybridize in all combinations in the laboratory, and many F_1 individuals have proved fertile in backcrosses (Blair 1963). Only *B. woodhousei* is sympatric to any extent with other species in the complex, and, although hybridization is common (as in *B. americanus* and *B. w.*

fowleri), partial temporal isolation and ecological isolation (breeding site and non-breeding habitat) have developed and distinctness is often maintained over large areas of geographic sympatry. *B. woodhousei* seems to warrant its status as a species distinct from the others of the *B. americanus* group by the criteria of Short (1969). In addition, Jones (1973) has presented evidence that reproductive isolation may have intensified in 30 years in one area of sympatry of *B. woodhousei* and *B. americanus*. However, Loftus-Hills (1975) has questioned aspects of this analysis.

The status of the other species is more problematical. The case for conspecific status of *B. a. americanus* and *B. a. hemiophrys*, two of the most morphologically distinctive taxa in the group, has been presented. The *B. americanus-terrestris* relationship has never been sufficiently analysed. *B. houstonensis* has been regarded as the most weakly distinctive of the group (Blair 1972). The geographic isolation of *B. houstonensis* and *B. microscaphus* is not sufficient in itself to warrant separate species status for each. Laboratory hybridization studies (Blair 1972), ecology and the occurence of natural hybridization of both *B. houstonensis* and *B. microscaphus* with *B. woodhousei*, suggest that if either were in contact with other *americanus* subgroup toads they would interbreed. It may be that the most pragmatic eventual classification to indicate relationships within the *B. americanus* subgroup will be to regard *terrestris, americanus, houstonensis, hemiophrys* and *microscaphus* as either allospecies or megasubspecies of *B. americanus* (or rather, of *B. terrestris* which is the oldest name, see Schmidt 1953). A choice between the two would be difficult but because of their similar interactions with *B. woodhousei* the latter may be best. Either would be consistent with the close relationships noted by many authors from many lines of evidence (Blair 1972) and place them into better evolutionary and zoogeographic perspective with other taxa of North American *Bufo*.

In part, a taxonomic decision on taxa pairs as closely related but distinctive as the pair analysed here is often coloured by the speculative opinion of the taxonomist concerned as to the past events influencing the present situation and eventual outcome of their interactions. Some assume that isolating mechanisms will inevitably develop and/or intensify (Remington 1968) and therefore the allocation of species-status is actually a prediction. Others regard any interbreeding as eventually breaking down the distinctiveness of present morphological differences with the eventual merging of the two forms. However, some zones of interbreeding along ecotones are known or assumed to remain for long periods without changing the taxa (Short 1970). The advantage of the Amadon and Short (1976) treatment is that it distinguishes this intermediate distinctiveness of the taxa and avoids the obscurity of conventional subspecies status (Rising 1970).

Because of the size and geographic extent of the samples used, the presumed objectivity of the discriminant analysis, and the repeatability of the measurements and scoring systems used, resampling in the future should be possible with results directly comparable to those presented. The weights generated should allow precise evaluation in 10, 25, 50 or more years of the then current status and perhaps provide an insight into exactly how, and if, the interbreeding and introgression affects future populations of these toads.

Obviously, the possible importance of such interactions in evolution could be in either adding additional variation for selection to act upon or as an intermediate step in eventual attainment of complete reproductive isolation. Only long-term studies, probably exceeding one or more human lifetimes will give sound assessment of such situations.

Until now, many laboratory techniques have been applied to problems of interspecific classification in toads (Blair 1972). Too little attention has been paid to intraspecific variation (for a notable exception see Guttman 1975). Although natural introgression has often been invoked to explain variable results within taxa, insufficient attention has been paid to documenting its existence and extent. As Blair (1972) has suggested for interspecific studies, a multidisciplinary approach to intraspecific relationships involving extensive analysis of geographic variation in cytogenetic and biochemical characters as well as additional morphological comparisons of the type presented here are needed.

Subsequent to this analysis, Green (1981) resampled a portion of the Trans-Canada transect and examined the variation through isozyme electrophoresis. He compared *B. a. americanus* hybridization with *B. a. hemiophrys* there with its hybridization with *B. w. fowleri* in southwestern Ontario. Although clearly obtaining results similar to the morphological analysis presented here, distinguishing that the two pairs are hybridizing

to a different extent, and recognizing that hybrid zones are an indication of genetic relationships, Green asserts that each of these taxa should all be treated taxonomically as species. The utility of the subspecies category is lightly treated as is the use of megasubspecies. In arguing that hybrid zones have little, or at best, only indirect influence on evolutionary change, and apparently accepting the principle that recognizable differences between populations demand a species-level distinction be made, he prefers to define a species on the subjective grounds of what a researcher can recognize as different. This approach obviously has appeal, particularly as in the present case when it attempts to rationalize the maintenance of taxonomic levels which are familar through long use in the literature. It seems however, too subjective, and an unsatisfactory manner of reflecting the dynamics of the relationships.

Green has deposited the specimens from his study at the National Museum of Natural Sciences, where they will be measured and analysed by the methods used here, and a direct comparison of the two approaches will be reported by the two of us when completed. We are agreed that the importance of these studies lies in examining such interactions in depth from many approaches and the question of the taxonomic arrangement one prefers should not obscure this.

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APPENDIX I: List of collections measured

Locality Number	Latitude (N) and Longitude (W)	Locality	Collection date	NMC catalogue number	Number of specimens ♂	♀
Reference sample: ***Bufo a. hemiophrys***						
Alberta						
1A	58°25′ 114°46′	Mikkwa River	04 June 1968	14045	8	1
2A	54°11′ 113°52′	2.4 miles [3.9 km] N on Highway 44 of Westlock	26 May 1965	8521	48	15
3A	54°24′ 113°29′	6.0 miles [9.7 km] SW on Highway 2 of Perryvale (turnoff)	27 May 1965	8527	36	0
4A	53°32′ 114°31′	0.5 miles [0.8 km] E on Highway 16 of Stony Plain	18 May 1965	8502	8	0
		0.5 miles [0.8 km] Ξ on Highway 16 of Stony Plain	20 May 1965	8505	47	0
5A	53°57′ 111°20′	3.3 miles [′ km] SW of St. Paul	05 May 1965	8547	27	0
6A	51°05′ 114°05′	3.2 miles [>.2 km] N and 1.6 miles [2.6 km] E of junction of 4th St. NE and NW Drive, Calgary	25 May 1966	10332	12	0
			20 May 1966	10325	3	0
			25 May 1966	10333	9	0
			Spring 1967	10755	12	0
7A	51°03′ 113°20′	2.0 miles [3.2 km] E on Highway 1 of Strathmore	29 May 1963	7115	14	0
		2.9 miles [4.7 km] E on Highway 1 of Strathmore	30 May 1963	7116	3	0
		Strathmore	30 May 1963	7117	3	0
8A	50°30′ 111°55′	2.2 miles [3.5 km] NW on Highway 1 of Brooks (turnoff)	20 May 1962	6021	11	1
		0.8 miles [1.3 km] NW on Highway 1 of Brooks (turnoff)	20 May 1962	6022	31	6
		0.5 miles [0.8 km] NW on Highway 1 of Brooks	20 May 1962	6023	26	3
Saskatchewan						
1S	53°13′ 105°53′	Shell River, 4.4 miles [7.1 km] W on Highway 3 of Prince Albert	21 June 1959	4049	4	3
			22 June 1959	4068	5	0
		Pond, 0.1 miles [0.2 km] E of Shell River on Highway 3	22 June 1959	4060	1	0
	53°12′ 105°46′	Prince Albert	21 June 1959	4052	1	0
		Prince Albert	23 June 1959	4072	7	0
2S	52°17′ 109°13′	6.5 miles [10.5 km] E on Highway 14 of Reward (turnoff)	13 May 1962	6007	40	3
3S	52°08′ 106°15′	14.5 miles [23.4 km] E on Highway 5 of Sutherland	28 May 1962	6038	44	7
	52°08′ 106°24′	8.2 miles [13.2 km] E on Highway 5 of Sutherland	28 May 1962	6040	44	0

APPENDIX I (continued): List of collections measured

Locality Number	Latitude (N) and Longitude (W)	Locality	Collection date	NMC catalogue number	Number of specimens ♂	♀
4S	50°05′ 102°28′	Leach Lake at Highway 9, 14.3 miles [23.0 km] S of Yorkton	16 June 1959	4033	22	1
5S	49°59′ 108°56′	8.3 miles [13.4 km] E on Highway 1 of Paipot (turnoff)	28 May 1963	7111	22	2
6S	50°04′ 108° 51′	3.3 miles [5.3 km] W on Highway 1 of Tompkins (turnoff)	16 May 1962	6014	36	7
7S	50°43′ 105°35′	NW end of Buffalo Pound Lake	07 June 1959	4007	27	6
8S	50°37′ 105°34′	Qu'Appelle River Valley, 1.8 miles [2.9 km] W and 15.2 miles [24.5 km] N of Belle Plaine	18 May 1961	5263	1	0
			19 May 1961	5267	13	0
			19 May 1961	5265	10	0
9S	50°29′ 103°24′	2.3 miles [3.7 km] E on Highway 1 of Sintaluta (turnoff)	15 May 1965	8496	74	3
Manitoba						
1M	53°43′ 101°15′	6–10 miles [9.7–16.1 km] S on Highway 10 of the Pas	7 June 1970	12252	14	0
2M	52°05′ 100°51′	8.0 miles [12.9 km] E on Highway 10 of Minitonas (turnoff)	9 June 1970	12256	20	0
3M	50°03′ 99°29′	0.5 miles [0.8 km] N of Highway 1 on Oberon (turnoff)	13 June 1960	4563	3	2
4M	50°11′ 98°19′	Delta Beach	14 June 1960	4565	5	14
5M	49°45′ 99°10′	Northeast section, Spruce Woods Forest Reserve	4 June 1962	6052	49	15
			20–21 July 1960	4608	34	26
Wyoming						
1WU	41°33′ 105°40′	Laramie River, 1.4 miles [2.3 km] SE on Highway 287 of Bosler, Laramie River Valley	26 June 1965	8610	6	0
				KRP*	30	3
North Dakota						
1DU	48°02′ 98°04′	41 miles [66 km] E of Devil's Lake	27 May 1957	3338	9	0
Reference sample: *Bufo a. americanus*						
Minnesota						
1MU	47°21′ 92°56′	4.0 miles [6.4 km] S on Highway 73 of Hibbing	26 May 1959	3994	30	0

*K.R. Porter Collection

APPENDIX I (continued): List of collections measured

Locality Number	Latitude (N) and Longitude (W)		Locality	Collection date	NMC catalogue number	Number of specimens ♂	♀
Ontario							
1O	51°17′	80°39′	Moosonee	5 June 1972	14797	18	1
2O	51°23′	80°28′	Whitetop Creek, 12.0 miles [19.3 km] NE of Moosonee	1 June 1972	14783	21	0
				2 June 1972	14791	39	0
3O	49°49′	92°58′	Beaver Creek, 6.0 miles [9.7 km] W on Highway 17 of Oxdrift	31 May 1970	12238	54	0
4O	48°06′	80°12′	Lake Kenogami	19 May 1963	7076	56	4
5O	46°19′	79°15′	10.5 miles [16.9 km] E on Highway 17 of North Bay	17 May 1963	7064	104	6
6O	45°22′	75°29′	1.0 miles [1.6 km] W of Carlsbad Springs	30 April 1963	6958	89	12
7O	42°19′	81°51′	0.5 miles [0.8 km] N of Rondeau Provincial Park, Kent County	17 April 1963	6916	47	3
8O	41°57′	82°31′	Point Pelee National Park, Essex County	5 April 1967	9684	10	7
Quebec							
1Q	53°35′	77°35′	Lac Attila, James Bay	17 July 1973	15851	14	1
2Q	51°52′	64°43′	"Mile 134 [Kilometre 215.7], N of Seven Islands" [Sept Îles]	10 June 1952	2265	14	0
				11 June 1952	2269	12	1
				17 June 1952	2277	1	0
				19 June 1952	2278	1	2
				21 June 1952	2279	1	0
				22 June 1952	2280	3	0
3Q	50°12′	66°23′	"Seven Islands" [Sept Îles]	19 May 1952	2237	24	2
				20 May 1952	2238	4	1
				21 May 1952	2239	1	2
				23 May 1952	2240	0	1
				24 May 1952	2241	1	0
				25 May 1952	2242	3	1
				29 May 1952	2251	0	6
				1 June 1952	2258	5	3
4Q	48°51′	64°12′	Cap-des-Rosiers	3 June 1971	13134	54	1
5Q	48°11′	67°09′	Routhierville	10 May 1953	2429	22	2
Prince Edward Island							
1P	46°13′	62°36′	0.6 miles [1.0 km] E of Roseneath	26 April 1959	3964	29	2
				10 May 1959	3988	20	0

APPENDIX I (continued): List of collections measured

Locality Number	Latitude (N) and Longitude (W)	Locality	Collection date	NMC catalogue number	Number of specimens ♂	♀
New Brunswick						
1B	46°11′ 64°47′	6.0 miles [9.7 km] N of Moncton	8, 9, 12 May 1959	8711	53	1
Nova Scotia						
1N	45°05′ 64°22′	1.0 miles [1.6 km] E of Wolfville	17 May 1964	8716	55	0
2N	44°47′ 63°09′	Musquodoboit Harbour	28 May 1964	8729	40	0
Eastern Manitoba *Bufo* Collection Stations						
The Northern Transect						
1	50°36′ 96°56′	3.0 miles [4.8 km] S on Highway 9 of Gimli	15 June 1970	12261	1	0
		2.0 miles [3.2 km] S on Highway 9 of Gimli	15 June 1970	12262	1	0
		2.0 miles [3.2 km] S on Highway 9 of Gimli	15 June 1970	12263	21	0
2	50°14′ 96°44′	1.0 miles [1.6 km] SW on Highway 59 of Libau (turnoff)	30 May 1968	11071	15	0
3	50°21′ 96°39′	2.0 miles [3.2 km] SW on Highway 59 of Scanterbury	13 May 1968	11077	13	0
4	50°16′ 96°30′	2.0 miles [3.2 km] N on Highway 12 of	24 May 1968	11033	1	0
			25 May 1968	11038	2	0
			25 May 1968	11039	11	0
			5 June 1968	11100	1	0
5	50°25′ 96°32′	Ditches along Highway 59 (at Patricia Beach turnoff) 5.0 miles [8.1 km] NE on Highway 59 of Scanterbury	31 May 1968	11072	23	5
		5.0 miles [8.1 km] NE on Highway 59 of Scanterbury	24 May 1968	11032	8	0
6	50° 27′ 96°32′	4.0 miles [6.4 km] S on Highway 12 of Grand Beach turnoff	24 May 1968	11034	30	0
7	50°33′ 96°42′	Lagoon, west beach, Grand Beach Provincial Park	21 June 1968	11196	42	0
8	50°33′ 96°41′	Lagoon extension, 1.0 mile [1.6 km] from E Park Gates, Grand Beach Provincial Park	21 June 1968	11197	24	0
9	50°37′ 96°32′	4.0 miles [6.4 km] N on Highway 12 of Grand Beach turnoff	25 May 1968	11037	18	0
10	50°37′ 96°26′	6.0 miles [9.7 km] W on Highway 11 of Ft. Alexander	5 June 1968	11099	31	2
11	50°37′ 96°21′	2.0 miles [3.2 km] W of Highway 11 of Ft. Alexander	5 June 1968	11098	12	0

APPENDIX I (continued): List of collections measured

Locality Number	Latitude (N) and Longitude (W)	Locality	Collection date	NMC catalogue number	Number of specimens ♂	♀
12	50°34′ 96°14′	1.0 miles [1.6 km] W on Highway 11 of Pine Falls	5 June 1968	11095	15	0
13	50°26′ 96°01′	1.0 miles [1.6 km] S on Highway 11 of Great Falls	5 June 1968	11094	3	0
14	50°19′ 96°04′	5.0 miles [8.1 km] N on Highway 11 of Lac du Bonnet	4 June 1968	11093	2	0
The Beausejour Transect						
15	49°57′ 97°00′	Bird's Hill Provincial Park	23 June 1968	12209	1	0
			23 June 1968	12210	12	7
16	50°05′ 96°31′	1.0 mile [1.6 km] N of Beausejour	15 May 1968	11017	21	2
17	50°04′ 96°25′	4–5 miles [6.4–8.1 km] E on Highway 44 of Beausejour	15 May 1968	11018	70	2
18	50°04′ 96°22′	7.0 miles [11.3 km] E on Highway 44 of Beausejour	15 May 1968	11021	16	0
19	50°04′ 96°19′	9.0 miles [14.5 km] E on Highway 44 of Beausejour	15 May 1968	11020	7	0
20	50°04′ 96°12′	14.0 miles [22.5 km] E on Highway 44 of Beausejour	15 May 1968	11019	48	8
21	50°05′ 96°04′	11 miles [17.7 km] S on Highway 11 of Lac du Bonnet	4 June 1968	11090	13	0
The Vivian Transect						
22	49°53′ 96°26′	1.0 mile [1.6 km] E on Highway 15 of Vivian	23 May 1968	11030	3	0
			26 May 1968	11040	35	0
23	49°53′ 96°23′	Broken River, 3.0 miles [4.8 km] E on Highway 15 of Vivian	22 May 1968	11029	29	5
24	49°53′ 96°18′	7.0 miles [11.3 km] E on Highway 15 of Vivian	22 May 1968	11027	7	0
24	49°53′ 96°16′	8.0 miles [12.9 km] E on Highway 15 of Vivian	22 May 1968	11028	49	4
25	49°55′ 95°55′	3.0 miles [4.8 km] N on Highway 11 of Elma	14 May 1968	11012	49	4
26	49°53′ 95°41′	10.0 miles [16.1 km] E on Highway 4 of junction of Highways 4 and 11, E of Whitemouth	13 May 1965	8483	24	0
27	49°51′ 95°28′	4.0 miles [5.4 km] E on Highway 44 of Rennie	14 May 1968	11011	34	35
28	49°51′ 95°24′	7.0 miles [11.3 km] E on Highway 44 of Rennie	14 May 1968	11008	55	11

APPENDIX I (continued): List of collections measured

Locality Number	Latitude (N) and Longitude (W)	Locality	Collection date	NMC catalogue number	Number of specimens ♂	♀
The Trans-Canada Highway Transect						
29	*49°38′ 96°59′*	3.0 miles [4.8 km] N on Highway 59 of Niverville (turnoff)	30 May 1970	12331	18	0
			31 May 1970	12232	3	0
			31 May 1970	12233	9	0
			31 May 1970	12234	2	0
			31 May 1970	12235	5	0
30	49°44′ 96°43′	Dufresne (turnoff at Highway 1)	8 June 1962	6086	6	0
31	49°49′ 96°34′	30.7 miles [49.4 km] W on Highway 1 of junction of Highways 1 and 11	8 June 1962	6104	10	0
		31.4 miles [50.6 km] W on Highway 1 of junction of Highways 1 and 11 W of Richer	14 May 1965	8487	19	4
		Dugout W of Lake Riviera turnoff, 3.0 miles [4.8 km] E of Ste. Anne (turnoff)	27 May 1968	11041	43	0
		31.25 miles [50.3 km] W on Highway 1 of junction of Highways 1 and 11	15 May 1969	11790	5	3
			27 May 1969	11811	10	2
			4 June 1969	11832	5	0
			5 June 1969	11847	2	0
		31.0 miles [50.0 km] W on Highway 1 of junction of Highways 1 and 11	19 April 1969	11736	1	0
			3 May 1969	11765	1	0
			7 May 1969	11773	3	0
			15 May 1969	11788	8	3
			3 June 1969	11831	3	0
			14 July 1969	12001	1	0
			18 May 1970	12205	2	2
			20 May 1970	12213	1	0
			21 May 1970	12214	6	0
			22 May 1970	12218	2	0
		30.0 miles [48.3 km] W on Highway 1 of junction of Highways 1 and 11	18 May 1970	12204	1	1
			22 May 1970	12215	1	1
			22 May 1970	12217	3	0
			12 June 1970	12257	1	0
		29.75 miles [47.9 km] W on Highway 1 of junction of Highway 1 and 11	15 May 1969	11789	0	3
			27 May 1969	11810	9	0
			27 May 1969	11812	1	0
			28 May 1969	11815	2	0
			5 June 1969	11840	3	0
32	49°49′ 96°26′	24.0 miles [38.6 km] W on Highway 1 of junction of Highways 1 and 11	23 May 1970	12219	2	0
			23 May 1970	12221	4	0
			23 May 1970	12222	14	0

APPENDIX I (continued): List of collections measured

Locality Number	Latitude (N) and Longitude (W)	Locality	Collection date	NMC catalogue number	Number of specimens ♂	♀
33	49°49′ 96°24′	22.6 miles [36.4 km] W on Highway 1 of junction of Highways 1 and 11	11 June 1961	5364	32	0
	49°49′ 96°23′	22.0 miles [35.4 km] W on Highway 1 of junction of Highways 1 and 11	22 May 1968	11026	14	0
34	49°49′ 96°18′	18.0 miles [29.0 km] W on Highway 1 of junction of Highways 1 and 11	19 May 1970	12208	13	0
			24 May 1970	12224	13	0
			24 May 1970	12227	11	2
35	49°49′ 96°17′	17.4 miles [28.0 km] W on Highway 1 of junction of Highways 1 and 11	7 June 1962	6064	74	0
		16.0 miles [25.8 km] W on Highway 1 of junction Highways 1 and 11, E of Richer	13 May 1965	8484	36	14
		Brokenhead River, 17.0 miles [27.4 km] W on Highway 1 of junction of Highway 1 and 11	11 May 1968	10993	6	0
			14 May 1968	11013	27	0
			21 May 1968	11025	17	2
		17.0 miles [27.4 km] W on Highway 1 of junction of Highways 1 and 11	14 May 1969	11786	10	11
			22 May 1969	11798	15	0
			23 May 1969	11800	2	0
			23 May 1969	11801	4	0
			26 May 1969	11805	0	1
			27 May 1969	11809	*23	13
			2 June 1969	11826	*4	0
			4 June 1969	11838	*4	0
			5 June 1969	11839	*16	3
			16 June 1969	11884	*7	0
			18 May 1970	12202	23	7
			23 May 1970	12220	0	2
36	49°49′ 96°09′	6.0 miles [9.7 km] E on Highway of Brokenhead River at Highway 1 (=11.0 miles [17.7 km] W on Highway 1 of junction of Highways 1 and 11)	25 May 1969	11802	24	0
37	49°49′ 96°08′	10.2 miles [16.4 km] W on Highway 1 of junction of Highways 1 and 11	11 June 1961	5361	9	0
38	49°49′ 96°07′	10.0 miles [16.1 km] W on Highway 1 of junction of Highways 1 and 11	12 May 1968	10995	4	0

* included in discriminant score mean and variance for this locality (35) but omitted from the univariate and ratio means which are based on the other 214 specimens from this locality.

APPENDIX I (continued): List of collections measured

Locality Number	Latitude (N) and Longitude (W)	Locality	Collection date	NMC catalogue number	Number of specimens ♂	♀
39	49°49′ 95°58′	3.0 miles [4.8 km] W on Highway 1 of junction of Highways 1 and 11	13 May 1968	10996	68	5
			6 May 1969	11775	14	0
			14 May 1969	11787	11	9
			17 May 1969	11794	6	0
			18 May 1969	11795	5	2
			3 June 1969	11830	7	0
			17 May 1970	12200	13	6
		2.0 miles [3.2 km] W on Highway 1 of junction of Highways 1 and 11	27 May 1968	11042	34	5
			26 May 1969	11807	16	5
40	49°49′ 95°49′	4.6 miles [7.4 km] E on Highway 1 of junction of Highways 1 and 11	10 June 1961	5350	35	0
41	49°49′ 95°58′	8.0 miles [12.9 km] E on Highway 1 of junction of Highways 1 and 11	13 May 1968	10997	7	0
42	49°34′ 95°27′	6.0 miles [9.7 km] W on Highway 1 of Falcon Lake (turnoff)	13 May 1968	10999	7	0
43	49°31′ 95°19′	Falcon Lake, Whiteshell Forest Reserve	05 June 1962	6054	114	2
		SW end Falcon Lake, Whiteshell Provincial Park	12 May 1965	8477	32	0
The Marchand Transect						
44	49°28′ 96°41′	8.0 miles [12.9 km] S on Highway 12 of Steinbach	27 May 1968	11044	74	0
			12 June 1970	12258	12	0
45	49°27′ 96°20′	12.0 miles [3.2 km] E on Highway 210 of Marchand	31 May 1968	11079	6	0
46	49°27′ 96°18′	4.0 miles [6.4 km] E on Highway 210 of Marchand	31 May 1968	11081	9	0
			01 June 1968	11088	16	0
47	49°12′ 95°20′	Refuse dump pond, Moose Lake, NW Angle Forest Reserve	16 June 1968	11133	5	0
			16 June 1968	11136	66	0
48	49°10′ 95°14′	Birch Point, Buffalo Bay, Lake of the Woods NW Angle Forest Reserve	17 June 1968	11146	17	0
			17 June 1968	11151	29	0
49	49°05′ 97°18′	3.0 miles [4.8 km] S on Highway 75 of Letellier	04 June 1970	12246	12	0
		3.5 miles [5.6 km] S on Highway 75 of Letellier	04 June 1970	12247	7	0
		4.0 miles [6.4 km] S on Highway 75 of Letellier	04 June 1970	12248	7	0
		1-2 miles [1.6-3.2 km] S on Highway 75 of Letellier	04 June 1970	12249	18	0
50	49°02′ 96°49′	4.0 miles [6.4 km] S on Highway 59 of Tolstoi	30 May 1970	12229	37	5

APPENDIX I (continued): List of collections measured

Locality Number	Latitude (N) and Longitude (W)	Locality	Collection date	NMC catalogue number	Number of specimens ♂	♀
51	49°41′ 96°21′	5.0 miles [8.1 km] NW on Highway 12 of Rat River	28 May 1968	11047	0	2
		3.0 miles [4.8 km] NW on Highway 12 of Rat River at Highway 12	28 May 1968	11048	55	4
52	49°12′ 96°17′	Just E on Highway 12 of Rat River at Highway 12	29 May 1968	11062	6	1
	49°12′ 96°16′	1.0 mile [1.6 km] E on Highway 12 of Rat River at Highway 12	29 May 1968	11065	39	9
53	49°11′ 96°15′	3.0 miles [4.8 km] SE of Rat River at Highway 12	30 May 1968	11067	9	0
54	49°06′ 96°09′	1.0 mile [1.6 km] NW on Highway 12 of Menisino (turnoff)	30 May 1968	11068	28	0
55	49°12′ 96°03′	7.0 miles [11.3 km] NW on Highway 12 of junction of Highways 12 and 89, N of Piney	28 May 1968	11051	14	0
56	49°13′ 96°12′	4.0 miles [6.4 km] NW on Highway 12 of junction of Highways 12 and 89, N of Piney	28 May 1968	11053	20	4
57	49°01′ 95°59′	3.0 miles [4.8 km] S on Highway 89 of Piney	30 May 1968	11069	21	0
		3.5 miles [5.6 km] S on Highway 89 of Piney	30 May 1968	11070	46	0
58	49°06′ 95°55′	3.0 miles [4.8 km] E on Highway 12 of junction of Highways 12 and 89, N of Piney	29 May 1968	11060	25	0
59	49°04′ 95°47′	1.0 mile [1.6 km] NW on Highway 12 of South Junction turnoff	29 May 1968	11055	35	0
60	49°02′ 95°39′	1.0 mile [1.6 km] W on Highway 12 of Sprague (turnoff)	29 May 1968	11056	69	3
61	49°01′ 95°25′	0.5 mile [0.8 km] E on Highway 12 of Middlebro	29 May 1968	11057	26	4
		Highway 12 at Middlebro turnoff	29 May 1968	11058	42	4

APPENDIX I (continued): List of collections measured

Latitude (N) and Longitude (W)	Species/Locality	Collection date	NMC catalogue number	Number of specimens ♂
Bufo woodhousei fowleri				
Ontario				
42°34′ 80°15′	Long Point, Norfolk Co.	19 June 1973	15972	19
Bufo woodhousei woodhousei				
Kansas				
39°19′ 99°14′	3.5 miles [5.6 km] SSE of Stockton, Rooks Co.	23 June 1965	8585	10
		23 June 1965	8591	7
Bufo boreas				
Alberta				
54°44′ 113°21′	2.0 miles [3.2 km] W on Highway 2 of Athabasca	28 May 1965	8530	60
Bufo boreas x *hemiophrys*				
Alberta				
54°09′ 113°52′	2.4 miles [3.9 km] N on Highway 44 of Westlock	26 May 1965	8523	1
Bufo cognatus				
Saskatchewan				
49°59′ 109°07′	8.3 miles [13.4 km] E on Highway 1 of Piapot (turnoff)	28 May 1963	7110	48

APPENDIX II: List of localities and collections for which male breeding calls were recorded.

Catalogue numbers given are the National Museums of Canada (NMC): lot number, followed by a dash and the individual specimen(s) number within that lot, followed (in parenthesis) by the tape catalogue number. Data used for each specimen is on file at the NMC in the Herpetology Section at the Museum of Natural Sciences on index card file by tape number.

Most specimens recorded were also used in the morphological analysis and localities are numbered in Appendix I with latitude and longitude, and mapped in Figure 2 and 3. A few localities from which recordings were obtained contained too few specimens to be included in the morphological analysis. The discriminant scores, however, were computed and have been used in the comparisons of morphology and call. Included in the call reference samples are some localities in southeastern Manitoba excluded from the morphological reference samples.

Locality	Number of specimens	Date	NMC catalogue numbers
Bufo a. hemiophrys			
*6–10 miles [9.7–16.1 km] S on Highway 10 of The Pas	7	7 June 1970	12252-1 to 6(68), -7(69)
*8.0 miles [12.9 km] E on Highway 10 of Minitonas turnoff	1	9 June 1970	12256-1(69)
*2.0 miles [3.2 km] S on Highway 9 of Gimli	8	15 June 1970	12263-1 to -8(70)
*3.0 miles [4.8 km] S on Highway 75 of Letellier	3	4 June 1970	12246-1 to -3(64)
*1–2 miles [1.6–3.2 km] S on Highway 75 of Letellier	17	4 June 1970	12249-1 to -4(64), -5 to -9(65), -10 to -14(66), -15, -16, -18(67)
*4.0 miles [6.4 km] S on Highway 59 of Tolstoi	7	30 May 1970	12229-1 to -7(54)
3.0 miles [4.8 km] N on Highway 59 of Niverville turnoff	12	30 May 1970	12231-1 to -5(55), -6 to -12(56);
	2	31 May 1970	12232-1, -2(57)
	4	31 May 1970	12233-1 to -3(57) -8(58)
	1	31 May 1970	12234-2(58)
	3	31 May 1970	12235-1, -3, -5(58)
*0.5 miles [0.8 km] N on Highway 75 of Glenlea	1	2 June 1970	12244-1(63)
1.0 mile [1.6 km] N on Highway 75 of Glenlea	3	1 June 1970	12243-1, -2, -4(62)
*4.0 miles [6.4 km] N on Highway 75 of Glenlea	1	1 June 1970	12241-1(61)
8.0 miles [12.9 km] S on Highway 12 of Steinbach	2	12 June 1970	12258-1, -2(70)
31.25 miles [50.3 km] W on Highway 1 of junction of Highways 1 and 11	2	15 May 1969	11790-1, -2(24)
	2	4 June 1969	11832-1, -2(40)
31.0 miles [49.9 km] W on Highway 1 of junction of Highways 1 and 11	3	3 June 1969	11831-1, to -3(39)
	5	21 May 1970	12214-2 to -6(49)
	2	22 May 1970	12218-1, -2(50)
30.0 miles [49.9 km] W on Highway 1 of junction of Highways 1 and 11	1	22 May 1970	12215-1(50)
	3	22 May 1970	12217-1, -2, -4(50)
	1	12 May 1970	12257-1(69)
29.75 miles [47.9 km] W on Highway 1 of junction of Highways 1 and 11	2	27 May 1969	11810-1, -2(36)
	1	27 May 1969	11812-1(36)
	2	28 May 1969	11815-1, -2(37)
	2	5 June 1969	11840-1, -2(42)

* specimens used in call discrimination (45 specimens).

APPENDIX II (continued): List of localities and collections for which male breeding calls were recorded.

Locality	Number of specimens	Date	NMC catalogue numbers
24.0 miles [38.6 km] W on Highway 1 of junction of Highway 1 and 11	2	23 May 1970	12219-1, -2(51)
	4	23 May 1970	1221-1 to -4(51)
	9	23 May 1970	12222-1 to -9(52)
Total *B. a. hemiophrys*	111		
Intermediate populations			
21.0 miles [33.8 km] W on Highway 1 of junction of Highways 1 and 11	2	29 June 1969	11922-1, -2(43)
19.0 miles [30.6 km] W on Highway 1 of junction of Highways 1 and 11	1	24 May 1970	12223-1(52)
	1	24 May 1970	12226-1(53)
18.0 miles [29.0 km] W on Highway 1 of junction of Highways 1 and 11	6	24 May 1970	12224-1, -2(52), -3 to -6(53)
	3	24 May 1970	12227-1 to -3(54)
	1	24 May 1970	12228-1(54)
17.0 miles [27.4 km] W on Highway 1 of junction of Highways 1 and 11	2	23 May 1969	11800-1, -2(30)
	1	23 May 1969	11801-1(31)
	9	27 May 1969	11809-1, -3(34), 4 to -9(35) -10(36)
	3	2 June 1969	11826-1 to -3(38)
	4	4 June 1969	11838-1 to -4(40)
	4	5 June 1969	11839-1 to -4(41)
	2	16 June 1969	11884-1, -2(42)
	23	18 May 1970	12202-1 to -5(45), -6 to -11(46), -12 to -17(47), -18 to -23(48)
Total intermediate *Bufo*	62		

APPENDIX II (continued): List of localities and collections for which male breding calls were recorded.

Locality	Number of specimens	Date	NMC catalogue numbers
Bufo a. americanus			
6.0 miles [9.7 km] E of Brokenhead River at Highway 1 (= 11 miles [17.7 km] W on Highway 1 of junction of Highways 1 and 11	5	25 May 1969	11802-1 to -5(33)
*2–3 miles [3.2–4.8 km] W on Highway 1 of junction of Highways 1 and 11	3	19 May 1969	11796-1 to -3(27)
	5	26 May 1969	11807-1 to -5(34)
*3.0 miles [4.8 km] W on Highway 1 of junction of Highways 1 and 11	7	6 May 1969	11775-1, -2, -3, -6, -7, -9, -12(23)
	1	12 May 1969	11783-1(24)
	1	16 May 1969	11793-1(24)
	5	17 May 1969	11794-1 to -5(25)
	5	18 May 1969	11895-1 to -5(26)
	8	17 May 1970	12200-1 to -8(44)
Ontario			
*6.0 miles [9.7 km] W on Highway 17 of Oxdrift	10	31 May 1970	12238-3 to -6(59), -7 to -12(60)
Total *Bufo a. americanus*	50		
Total *Bufo*	223		

* included in call discrimination (45 specimens)